MYCOLOGIA

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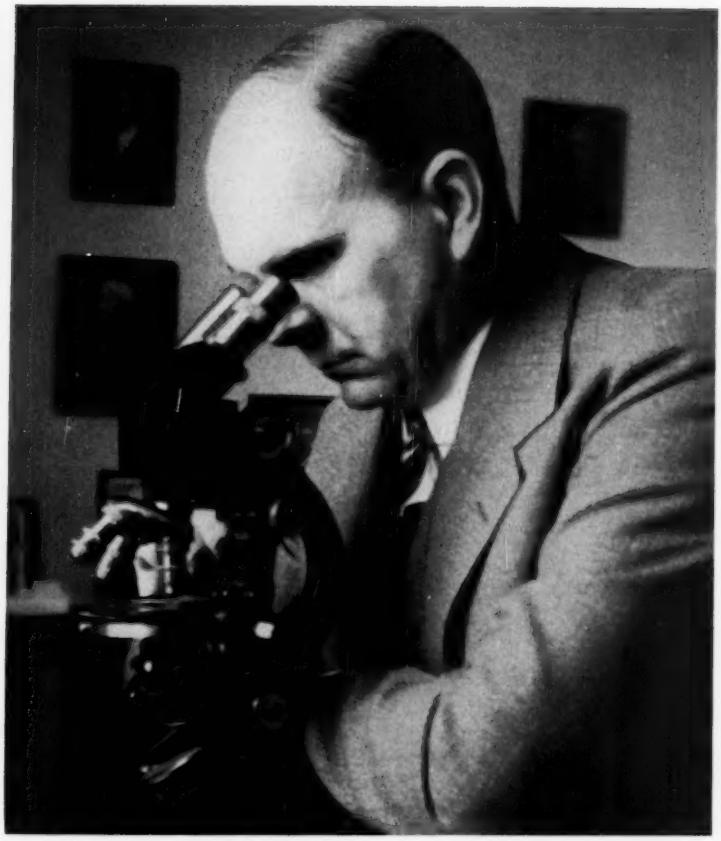

Harry Morton Fitzpatrick.

Photographed by his son Hugh in the summer of 1937, when he was 51 years of age.

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VOL. XLIII MAY–JUNE, 1951 No. 3

HARRY MORTON FITZPATRICK
1886–1949

M. F. BARRUS

(WITH THREE PHOTOGRAPHS)

Death came to Harry Morton Fitzpatrick on December 8, 1949, at his home on Bryant Avenue at Ithaca, New York, and he lies buried beside his son Harold in the family plot, selected by him, in East Lawn Cemetery not far from his home. Thus ended the career of a man who devoted fully forty years to the science of mycology, who was active in the organization of the Mycological Society of America, and who served as its secretary, president, and historian, as well as associate editor of MYCOLOGIA, its official publication. It is fitting then that this journal should contain an account of his life and of his contributions as a teacher and as a researcher. A memorial statement has been prepared by Dr. D. S. Welch which was read by him before the faculty of the New York State College of Agriculture and published in the "Necrology of the Faculty" of Cornell University. One of his former students, Dr. W. W. Ray, presented a paper on Professor Fitzpatrick's life which he read last year before the History and Philosophy Section of the Nebraska Academy of Sciences.

Professor Fitzpatrick was born June 27, 1886, in the small village of Greenwood, Indiana, ten miles south of Indianapolis. His father, James Edwin Fitzpatrick, and his mother, Addie Rowe (Watson) Fitzpatrick, lived in Greenwood and were married there on March 30, 1885. One other son, Otto, born July 1, 1888, resulted from this union. James Fitzpatrick was born in Johnson

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County, north of Franklin, Indiana, May 9, 1859, the son of Salem and Mary Evelyn (Shaffer) Fitzpatrick. Salem's father, James, was born in Kentucky and came to Johnson County in 1840 with his father, Dennis, who was born in Ireland. Addie Rowe (Watson) Fitzpatrick was born in Warren County near Hamilton, Ohio, September 11, 1860, the daughter of William Washington and Mary Ellen (Larkin) Watson. William's great-grandfather came to Pennsylvania from England. The Larkins came to Ohio from Virginia.

James Edwin Fitzpatrick was a poultry merchant for many years, and, in the pursuit of his business, moved his family from Greenwood to Effingham, Illinois, when his son Harry was four and a half years old; to Shelbyville, Illinois, when he was nine; and to Crawfordsville, Indiana, when he was about eleven. The parents lived at the latter place throughout the remainder of their lives and it was Harry's home town until he married.

When he was thirteen years old, Harry Fitzpatrick joined The First Baptist Church of Crawfordsville, much to the joy of his parents who were devoted church members. For the next nine years he was a regular attendant at church and Sunday school services, young peoples meetings, and even occasionally at Wednesday-night prayer meetings. After going to Ithaca, he attended church once or twice but never became a member. His withdrawal from church activities was due largely to a changed belief resulting from reading Darwin's works and other books in the field of natural history. Thereafter, his Sundays were spent for the most part in study, correspondence, and field trips. He favored the church life of his wife and children, however, and regarded the church as a great good for the community.

During his first three years at Crawfordsville, Fitzpatrick's school work was rather unsatisfactory. But his 8th-grade teacher stimulated his interest and he was soon leading his class in English, history, and human physiology. The latter subject was taught by a man trained as a zoologist and he interested his students in natural history and in such fossils as trilobites and crinoids that were found nearby. Fitzpatrick joined a boys' nature-study club, called The Open Window Club, sponsored by *The Chicago Tribune*, the members of which collected birds' eggs, butterflies, mineral

specimens, and other natural objects. These first years in Crawfordsville were perhaps his most care-free ones and he revelled with his companions in various boys' games, common to the period, and in other activities. He and his brother did chores at home to lighten the work of their mother, whose health began to fail at this time. He began "passing" the Indianapolis paper in Crawfordsville when he was thirteen and continued to do so throughout his high school and college years as an aid in meeting his expenses. In a contest for the *Indianapolis News* during the summer of 1904, he obtained the largest number of subscribers and thereby won a free trip to the St. Louis World Fair. This was his first trip away from home alone and was a most memorable experience.

During his high school days, he became very studious. He had a room of his own at home and there he spent many a long evening studying. He also read extensively the best sellers and much of the better English literature. Here he became possessed of a desire to attend college. Wabash College was located only a block away from his home at that time and it was natural that activities there should direct his thoughts toward a college career. He took botany during his freshman year in high school under Walter King. King was a good teacher and his influence led young Fitzpatrick to become deeply interested in science and to think of botany as a life work. While taking the course, Fitzpatrick collected two dozen slime molds and identified them under the supervision of King.

During the last two years of high school, Fitzpatrick's life was full of enthusiasm, ambitions, and friendships. He played basketball a great deal at the city YMCA where he was a member and where his contacts were numerous and important. There he formed perhaps the closest friendships of his life with a group of boys, all of whom later went to college. During the summers, he played tennis, a sport in which he became proficient in post-college days. While still a high-school freshman, he had met H. H. Whetzel during the latter's senior year at college. The two went bicycling on collecting trips to Covington Hill and the crinoid ravine. After Whetzel had gone to Cornell for graduate work, he continued to keep in touch with Fitzpatrick by correspondence and made a special effort to encourage him, as he did other promis-

ing students, and to direct his interest toward one or another field of botany.

Fitzpatrick was one of the top students of his high school graduating class and won a scholarship to Wabash College, which he entered in the fall of 1905. By taking courses in botany, he came under the influence of Professor M. B. Thomas, a former Cornell man and a most remarkable teacher. During the three years of his studies at Wabash, his mind was broadened by the well-balanced training of able teachers in an arts college at that time. Contacts with other students from various parts of the state and from other states brought him new points of view and concepts of life. While in college he maintained the high standard of scholarship of his high school days. During the summer of 1907 he spent ten days with Dr. J. C. Arthur working in his rust herbarium. Dr. Arthur, who had been impressed by the careful way Fitzpatrick had prepared his specimens, invited him to stay at his home while at Lafayette and, doubtless, had some influence in his selection of mycology as a life work.

Professor George F. Atkinson of the department of botany at Cornell University needed an assistant for the college year of 1908. After corresponding with Thomas, Whetzel suggested Fitzpatrick for the position, although he was in his junior year, as no seniors specializing in botany at Wabash were available there at that time. So highly did Whetzel and Thomas recommend Fitzpatrick that Atkinson decided to take him as an assistant and also arranged to have him complete his undergraduate work for an A.B. degree in the Arts College at Cornell. He continued as an assistant to Atkinson while taking graduate work during the next two years. His training under Atkinson prepared him well for the mycological work he was to do in future years. In 1911, he accepted the position Whetzel offered him as instructor to teach mycology in the department of plant pathology of the New York State College of Agriculture. Mycology had been taught in the department by Professor Whetzel and Dr. Donald Reddick since 1908 in a course called Etiology of Plant Diseases, a 4-hour course given throughout the year on the taxonomy and phylogeny of disease-producing organisms. Fitzpatrick assisted Whetzel with this course that first year but, during the college years of 1912

and 1913, he taught it alone and, after 1914, except for some summer courses in the early years, he had charge of all teaching work in mycology given by Cornell University until his death.

The year 1913 was an auspicious one for him because, during the course of it, he obtained his Ph.D. degree, was appointed assistant professor of plant pathology, and was married on September 15, to Florence Church Fenner of Ithaca, who became his devoted companion and helpmate throughout his life. As a result of this union, three children were born, Hugh F., Barbara, and Harold W., the latter dying under tragic circumstances when only 19 years old.

In 1913, the course in etiology was enlarged to cover more forms and extended over a period of two years. An additional 4-hour course was organized by Fitzpatrick to include slime molds and bacterial plant pathogens. He also assisted Reddick that year in teaching advanced plant pathology and, in 1917–18, he gave a course entitled Laboratory Methods in Plant Pathology. Aside from these two latter courses, his teaching was confined to mycology and he eventually settled on a long 2-year, or 4-semester, course for advanced students and a short 1-semester elementary course in Comparative Morphology of the Fungi, the latter given each alternate year. Both were 4-hour courses with two lectures and two laboratory periods each week. The advanced course covered the taxonomy and phylogeny of fungi. One year was devoted to Phycomycetes, Ascomycetes and Fungi Imperfecti and the alternate year to Basidiomycetes, and identification of fungi. The work in bacterial diseases of plants was dropped in 1917–18 but Myxomycetes were included for a time in the long course. In 1940 this was enlarged to a 5-hour credit course to give more time for laboratory work.

The number of students taking the courses varied from year to year, of course, but there were never more than 25 in the long course nor less than 10, most of whom were majoring in plant pathology. Practically all were graduate students, many from foreign countries. He also taught a 4-hour course, Comparative Morphology of the Fungi, equivalent to one term of the advanced course, in the Summer School of Biology at Cornell from 1923 until 1940 inclusive.

Fitzpatrick was thoroughly conversant with his subject and was able to express himself clearly and effectively both in lectures and in writing. He spoke distinctly and deliberately, commonly without notes, and often amazed his students by his ability to construct taxonomic keys, describe the characteristics of fungi under discussion, and cite authorities and dates from memory. He did not attempt to be humorous or to entertain his students although he did tell interesting stories about mycologists, in discussing their monographs, and explained how they became interested in their subject. His students were supplied with mimeographed lecture outlines, which were prepared with great care and in detail, and were revised from time to time. These outlines are a notable contribution in themselves and have been much sought after by other than his students. He made them available, usually at cost, to mycologists whom he thought would make good use of them. He finally, in 1930, published his outlines on the Phycomycetes as a text book," "The Lower Fungi. Phycomycetes," which is still the most complete work in this group of fungi, although a little out-of-date in a few orders. Some minor errors occur in it, as is true of most first printings, but it has received general commendation. Mycologists have regretted that he did not also publish as a companion volume at least his extensive notes on the Ascomycetes of which he had a comprehensive grasp. He originally had such a plan in mind but his studies convinced him that future research would bring about many changes in the taxonomic arrangement and that any publication of the group at the time would need extensive revision later.

The laboratory work in mycology will long be remembered by his students. An abundance of material was put out on Monday morning with a laboratory outline as a guide and was removed the following Sunday night. The student was supposed to have completed his work on the material within that time. The assignments were thought by most students to be too heavy, and they often worked on the specimens and the voluminous literature until late at night. The laboratory was always in use. Loafing and noise were not tolerated lest they distract the student from his work. Not all his students may have loved him but they respected him and those who have had occasion to use mycology in connec-

tion with their work are grateful for the thorough training they received under him.

Fitzpatrick made a practice of writing to prospective graduate students of plant pathology, outlining the work he gave in mycology and sought to interest them in taking his courses. He never pressed them to do so, however, and, if he thought they might not be interested in that field or unable to accomplish it with credit to themselves, he advised against it. Those who majored in mycology naturally received more attention outside of class periods than the others and he gave unsparingly of his time to direct them in their research problems and to make helpful suggestions. Every year he arranged to have taken a group picture of his students and himself. These were labeled, framed, and hung on the walls of the mycology laboratories. Many of these students have distinguished themselves in later years as mycologists or plant pathologists.

Fitzpatrick really liked his students, even those who did poorly, and tried to be friendly to them. Although not socially minded, he occasionally entertained students at his home. After his eleven o'clock lecture, he liked nothing better than to have lunch with those who cared to go and to engage in jokes and lively talk with them. Some of his students recall that, when one or two had joined him in lunch, he would take them afterwards for a short drive through the hills or along the lake shore. His relationship with his students was not as intimate in his later years but one should understand that he was under considerable mental strain and in poor health at that period of his life, and that this affected his attitude toward others. Not a few of his former students owe their position to the favorable recommendation he gave them. He wanted to say good things about a man in recommending him but was scrupulously honest in stating his opinions of a candidate's qualities. For this reason his recommendation carried considerable weight. He constantly sought the best places for his men and advised them when better ones were available. His correspondence throughout the years reveals letters from many students expressing their appreciation of the excellent training he gave them and of his helpfulness in obtaining a satisfactory position.

Although teaching was Fitzpatrick's most important contribution

Professor Fitzpatrick seated in front of cottage of Prof. F. C. Stewart at Seventh Lake, N. Y. August 1931.

to mycology and has had the greatest influence, he nevertheless has done significant, if not extensive, research in this field of science. His Ph.D. thesis (2) was naturally devoted to a study of certain fleshy fungi, since it was done under the direction of the most distinguished student of this group in America at that time. This paper gives evidence of the early interest of Fitzpatrick in tracing the origins of various forms and their relationship to one another, probably being influenced in this direction also by Atkinson who was deeply interested in the phylogeny of the fungi. This interest is evident in later papers dealing with other fungi. He also under-

Group of mycologists at camp of Prof. F. C. Stewart on Seventh Lake, N. Y., attending the Mycological Foray in August 1931.
Front: H. M. Fitzpatrick, C. W. Dodge, L. R. Hesler.
Rear: F. C. Stewart, Vera K. Charles, Jakob Lange, Gertrude Burlingham.

took taxonomic studies to clarify or to revise the systematic relationship of groups of fungi in which he was particularly competent.

Fitzpatrick was very meticulous and thorough in his work. On investigating a fungus or a group of fungi, he collected an abundance of material in all stages of development whenever possible. If the work involved monographing a group or the authenticity of the identification of a fungus, he went to great pains to obtain type or authentic material of all species studied, even though this required extensive correspondence with mycologists or curators of

herbaria in various parts of the world and long waits for the specimens to arrive. He visited herbaria that were readily accessible, sometimes spending several weeks, as at the Brooklyn Botanic Garden, The New York Botanical Gardens, and the Farlow Herbarium. Everything obtainable pertaining to the subject was read intensively. No effort was too great if necessary to clear up a disputed point, be it ever so minor. As a result, his published papers have an authenticity that makes them valuable. Most of his research dealt with ascomycetous fungi although, besides his early papers on fleshy fungi (**2**, **4**), he published his opinions on the relationship of certain Phycomycetes (**19**), produced a book on the lower fungi (**31**), and was co-author of a paper on an imperfect fungus (**45**). He always stated that he was not an authority on any group of fungi but his monographs on the Coryneliaceae (**14**, **50**) and the Nitschkieae (**18**) as well as studies on related fungi (**21**) have clearly described and have shown the relationship of the species of these groups. He probably knew them better than any other person. He left an incomplete manuscript on certain species of *Corynelia* (**60**) that he had intended to have published in MYCOLOGIA. In the course of his life he erected 1 new subfamily, 3 new genera, 11 new species, 1 new variety, and made 7 new combinations. He was also co-author in erecting 1 new genus, 2 new species, and 1 new combination. Most of these were made within the family, Coryneliaceae. Ciferri named a new genus, *Fitzpatrickia,* after him.*

In addition to his published research, Fitzpatrick wrote a popular article about gladiolus corm rot (**1**), a disease on which he worked when he first became associated with the department, popular bulletins and articles on mushrooms (**17**, **23**, **29**), several reviews of books on plant pathology and mycology (**5**, **7**, **27**, **46**, **56**), and several biographies of men who had done distinguished work on fungi (**11**, **28**, **43**, **55**, **59**). His bibliographical study of the Icones Pictae Specierum Rariorum Fungorum of C. H. Persoon (**54**) involved much time and an extensive correspondence. This included preparing and supplying to mycologists and a number of libraries parts of this rare volume that were lacking in all the

* Ciferri, R. A new genus of the subfamily Nitschkieae. Mycologia **20**: 29–30, fig. 1. 1928.

known copies available in this country. He also published notes and articles regarding the Mycological Society of America every year from 1932 until 1936. He gathered material relating to the history of mycology in America and in 1936 published an article (**49**) on the Historical Background of the Mycological Society in America and, later, another article (**53**) on its first twelve years. From the time Botanical Abstracts was first issued until its final volume, he abstracted articles on mycology for it, the first year as an abstractor, the second year as assistant editor on the morphology and taxonomy of fungi, lichens, bacteria, and myxomycetes, and as editor the following years of its existence. Professor Whetzel at his death in 1944 left an unfinished paper designed to characterize a new family, Sclerotiniaceae, that he had established and to give a synoptical treatment of its genera. Since this would mark the culmination of Whetzel's work in this group of fungi, Fitzpatrick magnanimously undertook the laborious task of assembling notes, drawings, and photographs and completing the article (**57**) without thought of credit for doing so. The excellent manner in which he accomplished this as well as his biography of Whetzel (**55**), written in his pleasing phraseology, brought him many compliments.

Professor Fitzpatrick, with other mycologists, was active in the founding of the Mycological Society of America at New Orleans on December 29, 1931, at which time he was elected its secretary-treasurer, an office which he filled for four years. He was then elected president and thereafter until his death was its historian. He was a member of the council during 1937 and '38. Before the Society was founded, he was for many years a member of the editorial board of MYCOLOGIA, which in 1932 became the official organ of the Society. During all these years, he devoted much of his spare time to the business of the Society and was influential in forming its policies. He published notes concerning its activities during this period and was especially active in the organization and conduct of mycological forays, sponsored by the Society, in which he took a special interest. He undertook the task of collecting the letters pertaining to the organization and other business of the Society, miscellaneous printed matter, group and individual photographs taken at its meetings, and other

material and having them bound into volumes for preservation in the Archives of the Society. Furthermore, he arranged for the permanent deposit of these materials for safe keeping in the Library of the New York Botanical Garden, where they may be consulted but not withdrawn.

Fitzpatrick, as professor of mycology, assumed the duties of curator of the Cornell plant pathology herbarium, although they materially increased his labor. This was fortunate because he applied the meticulousness characteristic of him to its building-up and maintenance. When the Atkinson herbarium was turned over to the department of plant pathology from the department of botany of the College of Arts and Sciences, he supervised the moving of the material and advised regarding the purchase of cases for storing it. When the Durand herbarium was purchased, he went to Minnesota and took charge of its shipment to Ithaca. He was greatly concerned over the safety of the Fairman herbarium willed to the University of Rochester and was instrumental in persuading the officials of that University to loan the herbarium to Cornell University for an indefinite period. It is now stored like the others in metal cases, protected from insects, and available for consultation. He recommended the purchase of desirable exsiccati and encouraged the collection of fungi by former students for deposit in the herbarium. There are 71 separate exsiccati and special collections, besides the general collection in the plant pathology herbarium. He prepared notes giving the nature of the material, its size and location in cases, and arranged for providing duplicate accession cards for all specimens. The herbarium was made accessible to all who came to examine specimens and, when possible, he sent those unable to visit the herbarium sufficient material for this purpose by registered mail. When type specimens were requested, he politely explained why they could not be sent but offered to examine the material himself or, in exceptional cases when material was sufficiently ample, to send a small portion on condition it be returned.

After becoming recognized as a competent mycologist, Fitzpatrick began receiving requests to identify fungi. He tried to discourage this, saying that he was not enough of a specialist to make such determinations, but he frequently examined material

sent him, sometimes identified it or gave suggestions as to its identity, and indicated specialists who might identify it positively.

Like many another professor, struggling with limited finances, Fitzpatrick took advantage of vacation periods to earn additional salary. During the summer of 1919, he worked on the potato-wart survey for the United States Department of Agriculture and, during July and August of 1920, was instructor in mycology at the University of Michigan. He gave a popular lecture on mushrooms and toadstools before the Brooklyn Institute of Arts and Sciences. From 1923 until 1940, except for two years, he taught mycology in the Cornell School of Biology for which he received compensation in addition to his regular salary. Advantage was taken of half-time sabbatic leaves on full salary, his first, in 1920–21, as a lecturer at Harvard University where his spare time was spent on research. He had planned to spend his second sabbatic during 1932 in Europe visiting herbaria in England, France, and other West-European countries. After about a month spent mainly in England and Holland, he changed his plans and returned home, much to the disappointment of those European mycologists who had wanted to meet him and of his friends at home who wanted him to have the advantage of many European contacts. His final sabbatic in 1948 was spent on an automobile trip with his wife that took them on a circle of the country to Florida, the West Coast north to Washington, and thence east back to Ithaca. On this trip, to which he had looked forward for years, he visited many of his former students located at experiment stations and colleges along the way and renewed his acquaintance with other Cornellians whom he met. This trip probably gave him the greatest enjoyment of his life although it may have been in a measure responsible for the decline of his health following his return.

In addition to being a charter member of the Mycological Society of America, Professor Fitzpatrick was also a member of the American Phytopathological Society, the Botanical Society of America, the American Association for the Advancement of Science, and the British Mycological Society. He declined membership in the New York Academy of Sciences for financial reasons. He was a member of the honorary societies Sigma Xi and Phi Kappa Phi. In the course of his duties he became chairman of

the organizing committee of the Mycological Club of the Northeast, 1919–20, which on his advice was not organized; member of the committee on nomenclature for the United States Bibliographical Committee (Mycology) representing Botanical Abstracts, 1920; executive secretary of the mycological section of the Fourth International Botanical Congress held at Ithaca, 1926; member of the sectional committee on fungi and fungous diseases of the Third International Congress of Microbiology held in New York, 1940; and member of the Research Club of Cornell University.

During his high-school and college days when he was active in basket-ball and tennis, Fitzpatrick was a tall, slender, and muscular fellow but, in after years, due perhaps to lessened physical activity, he became much heavier, weighing as much as 215 pounds. Instead of continuing his interest in sports, his exercise consisted largely of going on collecting trips with his students and sometimes with groups of mycologists. He made many motor trips with his wife, who was interested in the genealogy of her New England ancestors, throughout New England, eastern Canada, and, every year while his parents lived, to their home in Indiana. Fitzpatrick was devoted to his mother and, after he came to Cornell in 1908, arranged for her to visit him nearly every year while she lived. He was very proud of his family and took photographs of them frequently which he liked to show to his acquaintances. In his contact with others, especially in his later years, he seemed somewhat retiring and had few really close friends—friends with whom he could discuss personal matters freely. Yet, during social affairs, he was friendly and companionable. His correspondence through the years with his students, with scientists, and even with strangers, shows a remarkable interest in their affairs and a real effort to be helpful in his counsels to them. In his biographies of scientists, he showed a sympathy and rare understanding when recounting their lives. In 1944, he suffered a ruptured appendix and nearly died as a result. A few months later he had a second major operation for incisional hernia from which he recovered in 1946 but felt he would never again be able to do vigorous field work. He worried about his teaching and became very gloomy. After a time he seemed to recover and was able to attend to his duties and to carry on some investigational work. During this period he worked on

the article that was left unfinished and completed Whetzel's manuscript on the Sclerotiniaceae. He also wrote the splendid biography of Whetzel and his final one on Stewart. Then he suffered another relapse but seemed improved in health at the start of and during the long motor trip of 1948. It was evident, however, after his return that he was a changed man. He was dispirited and unhappy and, although he attempted to teach, most of that year was spent at home or at Clifton Springs Sanitorium. It seems likely that worry over his ill health and remorse over the death of his son were responsible in no small degree for his departure from this life in early December 1949.

His colleagues and friends who have known Professor Fitzpatrick for years feel now that they have never appreciated his real worth. It is certain that he was a superb teacher and that his research was so carefully and thoroughly carried out it will be dependable for years to come. His students will remember him as long as they live and will be grateful for the instruction they received from him. Those who appealed to him for help in organizing and directing mycological affairs, for information regarding the identity of fungi, or in more social or personal matters will recall that they did not ask in vain. His relatives called him Harry and his friends called him Fitz, to his students he was known as Prof Fitz and to most others he was Professor or Doctor Fitzpatrick. This indicates the affection and the respect with which he was held.

PUBLICATIONS

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3. The parasitism, biology and cytology of *Eocronartium typhuloides* Atk. Abstract of a paper presented at the sixth annual meeting of the American Phytopathological Society at Philadelphia, Pa., 1915. Phytopathology **4**: 407. 1914.

4. A parasitic species of *Claudopus*. Mycologia **7**: 34–37. *fig. 1. pl. 153.* 1915.
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11. George Francis Atkinson. Science n.s. **49**: 371–372. 1919.
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25. (Note concerning C. G. Lloyd.) Mycologia **18**: 284–285. 1926.
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37. Note. Mycologia **25**: 155. 1933.
38. The Mycological Society of America. Mycologia **26**: 108–112. 1934.
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41. (With B. O. Dodge and F. C. Stewart.) The Seventh Lake foray. Mycologia **26**: 377–378. 1934.
42. The Mycological Society of America. Report of the third annual meeting. Mycologia **27**: 225–226. 1935.
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44. The Mycological Society of America. Announcement for 1935 Summer Foray. Mycologia **27**: 327–328. 1935.
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46. (A review.) British Stem and Leaf-fungi, etc., by W. B. Grove. Science **83**: 106. 1936.
47. The Mycological Society of America. Summer Foray. Noteworthy collections. Mycologia **28**: 101–102. 1936.
48. The Mycological Society of America. Report of the fourth annual meeting. Mycologia **28**: 197. 1936.
49. Historical background of the Mycological Society of America. Mycologia **29**: 1–25. *figs. 1–5.* 1937. (With portrait.) Address of the Retiring President of the Mycological Society of America, given at the Atlantic City Meeting, December 29, 1936.
50. Revisionary studies in the Coryneliaceae. Mycologia **34**: 464–488. *figs. 1–43.* 1942.
51. Revisionary studies in the Coryneliaceae II. The genus *Caliciopsis*. Mycologia **34**: 489–514. *figs. 1–35.* 1942.
52. Note—Persoon, C. H. Icones Pictae Specierum Rariorum Fungorum. Paris et Strasbourg. 1803–1808. 4°. 64 pp. 24 tab. Mycologia **35**: 256–257. 1943.
53. The first twelve years of the Mycological Society of America. Mycologia **36**: 1–17. 1944. (With group photograph of mycologists in attendance at the first meeting at Atlantic City, Dec. 30, 1932.)
54. A bibliographical study of the Icones Pictae Specierum Rariorum Fungorum of Christiaan Hendrik Persoon. Mycologia **36**: 177–187. *figs. 1–4.* 1944.
55. Herbert Hice Whetzel. Mycologia **37**: 393–413. 1945. (With three photographs.)

56. (A review.) A manual of soil fungi, by Joseph C. Gilman. Scientific Monthly **61**: 238–240. 1945.
57. Statement, in H. H. Whetzel. A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. Mycologia **37**: 676; 678. Footnote 6. 1945.
58. (With W. W. Ray.) Some common edible and poisonous mushrooms. Cornell Extension Bulletin **386**: 1–16. *figs. 1–12.* 1946.
59. Fred Carleton Stewart. Phytopathology **37**: 687–697. 1947. (With portrait.)
60. Notes on *Coryneliа orcophila* (Speg.) Starb. and closely related species. *figs. 1–11.* [Incomplete manuscript.]

NOTES ON THE USTILAGINALES OF THE WORLD. V

GEORGE L. ZUNDEL [1]

USTILAGO BRAZILIENSIS Zundel, Mycologia **23**: 296. 1931.
Ustilago gregaria Zundel, Mycologia **23**: 296. 1931.

On *Panicum rivulare* Trin., Vicosa, Minas Geraes, Brazil, Agnes Chase, April 11, 1925; Juiz de Fora, Minas Geraes, Brazil, Agnes Chase, February 1925. After further study *U. gregaria* appears to be synonymous with *U. braziliensis.*

USTILAGO OXALIDIS Ell. & Tracy, Jour. Myc. **6**: 77. 1890.
Ustilago oxalidis var. *major* Dietel & Neger, Hedw. Beibl. **37**: 147. 1898.

On *Oxalis laxa* Hooker & Arn., Chile, F. Neger.

The type of the variety was loaned for study by the Botanical Museum in Stockholm. Its spores were not found to differ from those of the species.

Ustilago polygoni-alpini (P. Cruchet) Zundel, comb. nov.
Sphacelotheca polygoni-alpini P. Cruchet, Bull. Herb. Boiss. II. **7**: 247. 1908.

On *Polygonum alpinum* L., Col du cries, Canton du Valois, Switzerland, E. Mayor, August 8, 1907.

[1] Deceased March 10, 1950. Over a period of many years, Dr. Zundel brought together a comprehensive manuscript entitled The Ustilaginales of the World. The extent (997 pages) of this manuscript has precluded its publication to date and under present circumstances there is no immediate prospect of a publisher being found. It has seemed desirable, therefore, since Dr. Zundel had included some previously unpublished data in his general compilation, to take out such material for prompt publication in order both that it might be available and that he should have credit for his work to this extent at least. Some new combinations proposed by Zundel, but which in the light of present knowledge appear doubtful, have been omitted. The selection of the several items presented in this paper has been by Dr. Lee Ling.—John A. Stevenson.

Liro and Ciferri consider this species a synonym of *Ustilago bosniaca*, contending that the spores were not mature. The type specimen examined had mature spores but had no characters of a *Sphacelotheca*.

Ustilago sanctae-catharinae Zundel, nom. nov.

Ustilago occulta P. Henn. Hedwigia **36**: 212. 1897. (Not *U. occulta* Rab. 1844, on *Secale cereale* L.)

On *Andropogon* sp., St. Catharina, Brazil.

The name *Ustilago occulta* (Wallr.) Rab. was used in Rabenhorst's Handbuch **1**: 4. 1844 and in Klotz. Herb. viv. Myc. 1898, for *Urocystis occulta* (Wallr.) Rab. This makes it necessary to change the name of this species.

USTILAGO SHASTENSIS Zundel, in Cooke, Mycobiota N. Amer. No. 63. 1940.[2]

Sori destroying the individual axillary flowers, protected by the floral bracts, ovate, about 2 mm. diam., spore mass reddish-brown, semi-agglutinated; spores globose to subglobose or broadly ellipsoidal, often somewhat irregular, chiefly 7–10 μ long, light rosaceous-brown to almost hyaline, finely reticulate but not winged.

On *Polygonum shastense* Brew., Mt. Shasta, California, Wm. Bridge Cooke, Herb. No. 13381.

Sorosporium panici-carthagenensis (Speg.) Zundel, comb. nov.

Ustilago panici-carthagenensis Speg. Anal. Mus. Nac. Buenos Aires **6**: 207. 1899.

Sori in the inflorescence, concealed by the leaf-sheath, swollen, globose, 2–5 mm. diameter, hard, compact, spore-mass granular; spore-balls broadly ovate, opaque, dark brown, permanent, composed of many spores, 70–125 μ long; spores globose to ellipsoidal, bright olivaceous-brown, 8–10 μ diameter, smooth.

On *Panicum carthaginense* Sw. Cerro de Montevideo, Uruguay, 1882–91, J. Arecharaleta and C. Spegazzini.

[2] As *U. shastense*.

Sorosporium stiparum (Speg.) Zundel, comb. nov.

Ustilago stiparum Speg. Anal. Mus. Nac. Buenos Aires **19**: 288. 1909.

Sori destroying the inflorescence, long-linear, 4 cm. or more in length, covered by a delicate brown membrane which breaks up in long strips exposing a dark-brown, granular spore-mass intermixed with long, dark brown shreds; spore-balls chiefly ovate, occasionally oblong, opaque, composed of many spores, 50–78 μ long; spores globose to subglobose or broadly ellipsoidal, olivaceous-brown, 7–10 μ in diameter, smooth.

On *Stipa* sp., Lujan de Cuyo, Mendoza, Argentina, Jan. 1908, C. Spegazzini.

Urocystis alaskana Zundel, sp. nov.

Soris hypophyllis praecipue per nervos, pustulatis, elongatis, 3–6 mm. longis, primum textura hospitis tectis, ea rumpenti et massam fuscam pulverulentam sporarum detegenti; glomerulis sporarum irregularibus, 24–46.5 × 19.5–40.5 μ, e sporis fertilibus 1–5 compositis, ex parte cellulis sterilibus pallide olivaceis, subglobosis vel oblongis, 6–15 × 6–12 μ circumdatis; sporis intense rubro-brunneis, subglobosis usque angularibus, glabris, 13.5–23.5 × 12–18 μ.[3]

Sori on the under surface of the leaves, chiefly along the veins, pustulate, elongate, 3–6 mm. long, at first covered by the host tissue which ruptures exposing a dark powdery spore-mass; spore-balls irregular in shape, 24–46.5 × 19.5–40.5 μ, consisting of 1–5 fertile spores, partially surrounded by light olivaceous, subglobose to oblong sterile cells, measuring 6–15 × 6–12 μ; spores deep reddish brown, subglobose to angular, smooth, 13.5–23.5 × 12–18 μ.

On *Boykinia richardsoni* Gray, Nome, Alaska, Aug. 18, 1939, Dow V. Baxter.

Doassansia morotiana Zundel, nom. nov.

Doassansia intermedia Morot, Jour. de Bot. (Paris) **9**: 471. 1895. (Not *D. intermedia* Setch. 1894.)

On *Echinodorus ranunculoides* (L.) Engelm., Cholot, Maine-et-Loire, France.

[3] Latin diagnosis prepared by Edith K. Cash.

Note: George W. Fischer anticipating the publication in 1950 or earlier of the Zundel manuscript referred to in footnote [1] has used in his recent book, *The Smut Fungi* (Ronald Press, 1951) a number of binomials which he credits to Zundel as new combinations or as a new species, on the basis of the latter's usage in the manuscript in question. Eight such cases are involved and the reasons for not including the suggested new binomials in this paper follow: giving in each case the pertinent page in Fischer's book.

Page 25. *Melanopsichium emodensis* (Berk.) Zundel. Ling (Mycologia **41**: 257–258. 1949) has reported studies by himself and others clearly indicating that the fungus should remain in the genus *Ustilago*.

Page 30. *Sorosporium aristidae-cyananthae* (Bref.) Zundel. Pavgi and Mundkur (Indian Phytopath. **1**: 111. 1948) have placed this species in *Sphacelotheca* and Ling, who has studied authentic material, concurs.

Page 40. *Sphacelotheca hydropiperis* var. *columellifera* (Tul.) Zundel should be *S. hydropiperis* var. **berkeleyana** (Tul.) Zund. comb. nov.

Page 42. *Sphacelotheca pappophori* (Pat.) Zundel. This combination was also made previously by Zundel, Bothalia **3**: 300. 1938.

Page 110. *Urocystis hepaticae-trilobae* (DC.) Zundel and *U. hordei* (Cif.) Zundel. These transfers from *Tuburcinia* are in order should *Urocystis* be conserved against *Tuburcinia*, but there is no immediate prospect of such action and until this action is taken such transfers have no standing under the Rules of Nomenclature.

Page 113. *Urocystis ranunculi-auricomi* (Liro) Zundel. The same comment applies to this transfer.

Page 204. *Ustilago underwoodii* Zundel. Indicated as a new species by Zundel in his manuscript but actually published by him in Mycologia **34**: 124. 1942.—John A. Stevenson.

NOTES ON THE MORPHOLOGY OF THE GENUS HEMILEIA

K. S. GOPALKRISHNAN [1]

(WITH 7 FIGURES)

This paper records the results of an examination of thirty-two species of *Hemileia*. Morphologically, three distinct types of sori are recognized, and it is suggested that this diversity may be used as the basis for a more accurate taxonomic treatment of the genus, although the validity of the existing species is not taken into consideration in this study.

Hemileia vastatrix Berk and Br., the subject of a large number of papers, on account of its importance as the cause of the severe leaf disease of coffee in the Orient, is the type species of the genus *Hemileia*. This genus was erected in 1869 by Berkeley (**2**) on the basis of rust-infected coffee leaves sent to him by Thwaites from Ceylon. According to him the two main distinguishing characters of the genus were, (1) the stomatal sporulating habit, and (2) the characteristically reniform urediospores which were echinulate dorsally and smooth ventrally. These two characters were supplemented by Marshall Ward's (**15**) discovery of single-celled, smooth and round or napiform teliospores which germinated without rest. The latest taxonomic treatment of the genus as a whole is that of the Sydows (**11**) in which twenty-three species are considered, and for thirteen of these the teliospores are not recorded. Although the genus has now over forty species, in the case of over half the number the teliospores are unknown. Many species thus have been included in the genus merely on the basis of the form

[1] The author records his deep debt of gratitude and appreciation for all the help and inspiring guidance given by Dr. G. B. Cummins of the Arthur Herbarium, Purdue University, Indiana, during the course of this investigation. He is grateful to Mr. J. A. Stevenson of the U.S.D.A. for lending some of the specimens, and to the authorities of the Arthur Herbarium for the facilities given during the course of this study. The study was made possible by the J. N. Tata Endowment, Bombay, India.

of the urediospores, which is by no means constant, and the superstomal nature of the sorus. In spite of the fact that this last character is important it has rarely been accurately described.

Specific distinctions are difficult to recognize from published descriptions. Frequently what really may be only a new record for an area or a different host species is given a specific rank. This is difficult to understand when *H. vastatrix, H. woodii* Kaler and Cke., and *H. canthii* Berk and Br. occur on several species of their respective host genera while there are three different species of *Hemileia* on three closely related species of *Strophanthus.* Spore size, which is often the basis of species distinction, is variable and overlapping. These instances indicate that specific rank has been given to what are possibly physiologic races, such as are known for *H. vastatrix* (**9**). The above anomalies indicate the desirability of a critical study of the genus.

Host range. Species of *Hemileia* occur on both monocots and dicots and in the latter parasitize widely different families. Distribution of the various species of *Hemileia* according to the families of host plants is given in table 1.

TABLE 1

HOST RANGE OF THE GENUS *Hemileia*

Host family	No. of *Hemileia* spp. recorded	No. of types seen	Host family	No. of *Hemileia* spp. recorded	No. of types seen
Capparidaceae	1	1	Verbenaceae	1	1
Euphorbiaceae	1	1	Hypericaceae	1	1
Araliaceae	1	1	Rubiaceae	16	5
Oleaceae	3	2	Dioscoreaceae	1	1
Apocynaceae	9	4	Orchidaceae	4	2
Asclepiadaceae	5	2			

Materials and methods. Material studied was all from dried specimens in the herbarium. Free-hand as well as microtome sections were made for study. The free-hand sections were mounted in a temporary medium and often lightly stained with cotton blue for better differentiation. Microtome sections were made by the paraffin method, using butyl alcohol for infiltration (Zirkle's method), and differentially stained either according to Lepik's method (**6**) or with the Iron-Hematoxylin-Orange G combination.

SPERMAGONIA AND AECIA

Spermagonia and aecia are unknown for the genus. Beginning with Marshall Ward, who found the teliospores germinating in situ, all those working with the coffee rust have believed that *H. vastatrix* must be heteroecious. The fact that well-formed basidiospores do not reinfect coffee supports this view. Various workers (**4, 10, 13, 14**) have tried to connect the coffee rust with several unconnected aecia, notably those on *Pavetta* and *Vangueria*. The results from these trials were all negative. The writer, working for the coffee industry, made many series of cross inoculations with various aecia occurring on angiospermous hosts round about the coffee areas of Mysore and Coorg. The inoculations were made in the usual manner using coffee leaves of varying age, from three days to four weeks. Aeciospores for inoculation were collected fresh and part of each collection was allowed to germinate prior to inoculation while the other part was inoculated directly. Both incised leaves and potted seedlings were used for these inoculations. Not only were the aeciospore inoculations carried out on coffee leaves, but basidiospore inoculations were also carried out on the aecial hosts tried. In addition basidiospores were inoculated in each case on the coffee leaves themselves to determine the non-autoecious nature of the rust. The following aecia were tried for these experiments: *Aecidium eleagni latifoliae* and *Aecidium* species on *Randia dumatorum*, *Vangueria spinosa*, and *V. infausta*, *Plectronia didyma*, *Pavetta indica*, *Dioscorea bulbifera*, *Tabernaemontana indica*, *Loranthus longiflorus*, *Strobilanthes dalhousianus*, *Selacia indica*, and *Smilax* sp. All these aecia were found around coffee growing areas. All the inoculations gave negative results. The same was true of the uredial inoculations which included those species of *Hemileia* occurring in the neighborhood of the coffee areas in India. In these latter experiments emphasis was laid on the species of *Hemileia* occurring on rubiaceous hosts. The results thus confirm the findings of previous workers (**4, 10, 13, 14**).

UREDIA AND TELIA

With the exception of a few species the genus is foliicolous and hypophyllous. The exceptions are, the report of a race of *H.*

vastatrix on the fruits of *C. liberica* by Thirumalachar (**13**) and the occurrence of amphigenous sori in *H. Holstii* Syd., *H. paveticola* Roger and Moubl., and *H. mildbraedii* Syd., which are reported to occur on inflorescences and petioles. Rarely *H. vastatrix* has been collected on the upper surface of leaves and on petioles and pedicels.

Teliospores usually occur in the uredia with or following the urediospores, or if in separate sori the telia are morphologically similar to the uredia of the species. The teliospores are pedicellate, smooth, single-celled, thin-walled, and germinate immediately in all the species. They are usually smaller than the urediospores. A survey of the shape of the teliospores indicates that they are usually of two types, either rounded and napiform or highly angular and deeply lobed. The promycelium is four-celled and measures from 45 to 75 μ in length. Each cell bears a sterigma with the basidiospore. Prior to germination the two nuclei in the teliospore fuse, as has been reported by Thirumalachar (**13**).

Symptoms and infection. The general symptom of infection with the rust is the yellowing of the leaf, which may result in premature defoliation. The infection is of three general types: (1) The diffusely spreading type entirely covering the lower surface of the leaf, imparting to it a dusty powdery appearance. This is typical of *H. jasmini* K. & R., *H. hansfordii* Syd., and *H. Scheffleri* Syd.; (2) the pustular type as represented by *H. mysorensis* Thirum. and Gopal., and *H. evansii* Syd., with non-coalescent, comparatively large, ochraceous sori; (3) the infection of *H. vastatrix* type with large numbers of pustules coalescing to form a circular patch which increases centripetally, the younger infections appearing at the periphery and the central part gradually dying off. Often a few such patches may coalesce so that frequently the lower surface of the leaf is fully covered by two or three large patches. In the first two types the upper surface of the leaf shows little indication of the severe infection on the lower surface, while in the last type a clear yellow area corresponding to the infected patch on the lower surface is discernible. There is a strong correlation which will be referred to later between the type of microscopic infection and the type of sorus.

MORPHOLOGY OF THE SORUS

A critical examination of the available species indicated three important types of sori. The distinction between them can readily be made out in free-hand sections; they are distinct both in their origin and in their differentiation.

1. The subepidermal type, as represented by *H. evansii.*
2. Superstomal type "A" as typified by *H. vastatrix.*
3. Superstomal type "B" as in *H. jasmini.*

It will be noticed that types 2 and 3 are both superstomal. The cardinal point of difference between the two lies in the formation of the sorus and this is very clear. In the *H. vastatrix* type many horizontal hyphae cluster together in the substomal space and form fascicles which pass through the stomatal opening and produce spores either directly at the tips of hyphae or on specialized basal cells bearing sterigmata. In the *H. jasmini* type the contributing hyphae form a basal concentration usually around the bundles. There are rarely more than three such hyphae forming a swollen bulbous structure in the substomal space. This bulbous structure gives rise either to basal cells with sterigmata or to hyphae which bear spores at their tips. A detailed description of one species for each type follows and a synopsis of the species studied is given in table 2.

1. *The subepidermal type: H. evansii* Syd. is chosen as the type for this, since its sori are clearly subepidermal. The study was based on Doidge's collection (*no. 6635*). The uredia are hypophyllous and loosely grouped in yellowish spots up to 5 mm. in diameter, the spots visible on both sides of the leaf surface. Individual sori attain a size up to 0.4 mm. in diameter, are pulverulent, cushion-shaped and ochraceous. Sections indicate that the intercellular hyphae are extensive and fine and form haustoria profusely. In the substomal space these hyphae become highly tangled and form a basal hymenial layer of angular cells. The hyphae as well as this basal layer of cells are binucleate. As the tangled mass of hyphae increases in volume more and more of the mesophyll cells are destroyed and the remnants of these are seen at the margin of the sorus. The diameter of the sorus at this time is about 150 to 180 μ. Fascicles of dikaryotic hyphae arise from the basal layer

and occupy the space of several host cells in addition to the substomal space. These erect hyphae break through the stomata and displace five to six epidermal cells which fall back on themselves as in other subepidermal types of sori. The guard cells are com-

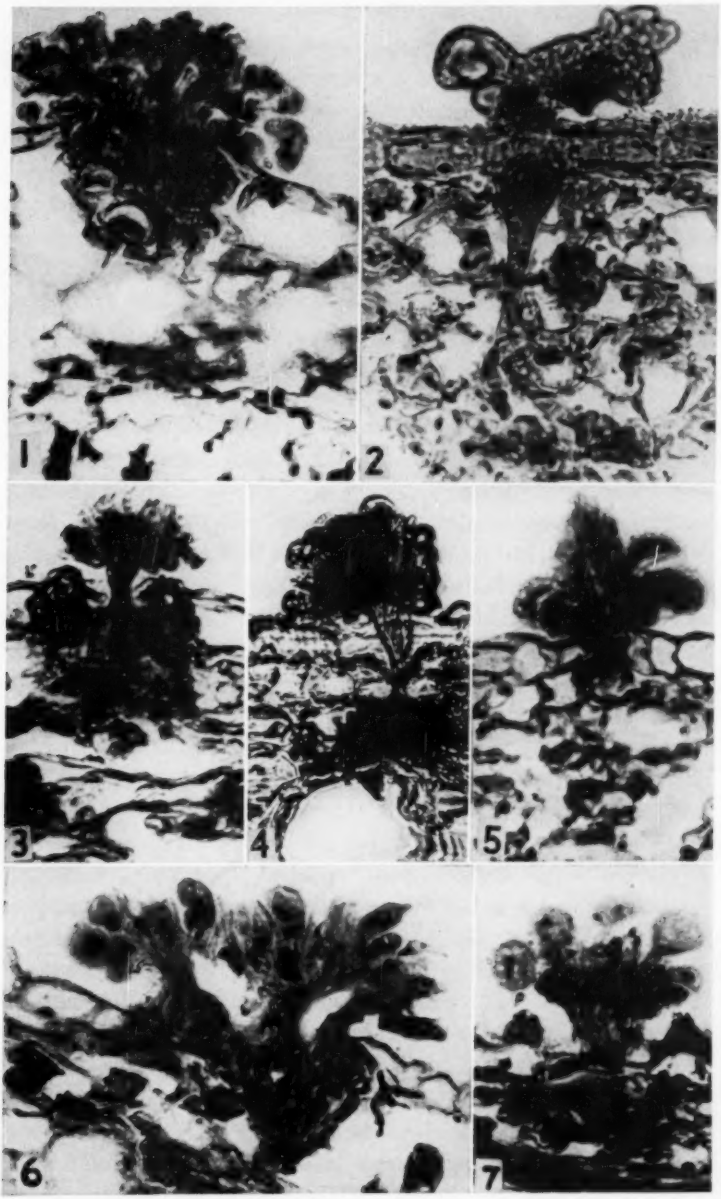
TABLE 2

UREDOSORAL MEASUREMENTS OF THE SPECIES STUDIED
(Measurements in Microns)

Sr. No.	*Hemileia* species	Host family	Height	Breadth		
				Stomal	Substomal	Superstomal
	Type 1: Subepidermal					
1	*H. evansii* Syd.	Rubiaceae	100–120	85–120	130–160	130–160
2	**H. holstii* Syd.	Rubiaceae	100–130	150–400	150–500	150–500
3	**H. fadogiae* Syd.	Rubiaceae	50–60	45–65	90–100	85–95
4	*H. smalii* Wakef. & Hansf.	Apocynaceae	55–65	40–60	30–40	60–70
5	**H. deightonii* Syd.	Apocynaceae (FIG. 1)	100–160	45–60	75–85	90–110
6	**H. mysorensis* Thirum. & Gopal.	Asclepiadaceae	125–155	45–55	100–120	70–90
	Type 2: Superstomal "A"					
7	*H. vastatrix* Berk & Br.	Rubiaceae	30–40	5–10	27–35	35–40
8	*H. canthii* Berk & Br.	Rubiaceae	60–170	6–10	30–50	35–45
9	*H. woodii* Kaler & Cke.	Rubiaceae	30–40	6–12	50–69	30–35
10	*H. gardiniae thunbergiae* Moubl. & Roger	Rubiaceae	60–80	6–12	30–35	35–50
11	**H. oxyanthi* Cumm.	Rubiaceae	75–90	8–10	17–25	15–20
12	**H. scholzii* Syd.	Verbenaceae	45–55	3–5	30–35	35–45
13	**H. harunganae* Cumm.	Hypericeae	35–50	6–10	17–25	25–35
14	*H. wrightae* Syd.	Apocynaceae	45–55	3–8	20–35	25–40
15	**H. buntungii* Wakef. & Hansf.	Apocynaceae	49–56	3–8	17–25	25–30
16	**H. chlorocodonis* Syd.	Asclepiadaceae	50–65	5–8	30–45	27–34
17	**H. scitula* Syd.	Asclepiadaceae	60–70	5–10	30–35	30–35
18	*H. oncidii* Syd.	Orchidaceae	65–70	3–8	17–25	25–30
19	*H. americana* Massee.	Orchidaceae	85–95	3–5	34–48	40–50
20	**H. dioscoreae aculeatae* Syd.	Dioscoreaceae	40–50	3–6	35–45	35–50
21	**H. antidesmae* Syd.	Euphorbiaceae	45–55	15–20	45–55	35–45
22	*H.* on *Aristolochia*	Aristolochiaceae	50–55	3–8	20–25	?
23	**H. phajii* Syd.	Orchidaceae	(Could not be made out definitely)			
	Type 3: Superstomal "B"					
24	**H. jasmini* K. & R.	Oleaceae	50–60	3–8	27–30	27–30
25	*H. hansfordii* Syd.	Oleaceae	65–75	3–5	30–35	35–45
26	*H.* on *Alafia*	Apocynaceae	45–50	3–5	30–35	35–45
27	**H. strophanthi* Racib.	Apocynaceae	50–85	6–8	30–40	30–40
28	**H. holerrhenae* Syd.	Apocynaceae	30–40	3–6	27–35	25–35
29	**H. Schefflerí* Syd.	Capparidaceae	50–60	6–10	25–30	25–30
30	**H. rutidae* Cumm.	Rubiaceae	35–45	3–6	27–35	25–35
	Intermediate Types					
31	*H. paveticola* Roger & Moubl.	Rubiaceae	55–65	30–35	30–35	30–35
32	*H. jahnii* Syd.	Apocynaceae	30–40	17–25	44–50	30–35

* Study based on type specimens.

Note: Cases where the specific names are omitted probably represent new species.



FIGS. 1–7. Sori of some species of *Hemileia*. 1. *H. deightonii*. 2. *H. jasminii*. 3. *H. vastatrix*. 4. *H. oncidii*. 5. Basal cells of *H. jasmini*. 6. *H. fadogiae*. 7. *H. scitula*. All × 400.

pletely destroyed. These hyphae branch profusely and cut off a typical reniform or rounded urediospore. Some of the spores are borne on short upright branches directly from the hymenial layer and they are clearly beneath the epidermal level. On account of this and the fact that a good length of the epidermis is ruptured in the formation of the sorus, this type is rightly called subepidermal. *H. holstii* Syd., another species belonging to this group, far exceeds *H. evansii* in the measurements of its sorus, reaching a breadth of 500 μ.

2. *Superstomal "A" type: H. vastatrix* Berk. & Br. is taken as representative of this type. Symptoms and infection of this rust have been so well described by previous workers (**13**, **14**, **15**) that discussion is out of place here. The sorus of either the uredium or the telium is formed by a group of intercellular hyphae running along the epidermis and almost next to it, forming a cluster or fascicle in the substomal space. There may be a single fascicle, resulting in a very small sorus, or there may be a group of fascicles protruding through the stoma. Neither the guard cell nor the adjacent epidermis is ruptured. Sporulation occurs only outside the host by means of specialized basal cells which develop sterigmata, or the spores may be borne directly at the tips of individual hyphae (FIG. 3). A similar but a more striking sorus is found in *H. oncidii* Syd. (FIG. 4) and *H. scitula* Syd. (FIG. 7) where, because of a very thick cuticle (10 μ), these fascicles of sporogenous hyphae are quite long and distinct. There appears to be no fusion between the adjacent hyphae. Although the guard cells are not destroyed, when the sorus is mature the stomatal opening is blocked with the sporogenous hyphae. In *H. canthii* and *H. woodii*, the hyphae in the substomal space swell up to form bulbous septate parts which separate and even develop echinulations and form what is termed by Thirumalachar (**13**) an internal sorus. This, however, is not a rule and may be due to environmental factors. Measurements of the superstomal sori vary from 4 to 12 μ at the stomal level and in the instances where they exceed this range there is a distention of the stoma. The distinctive feature of this type is the non-destruction of the guard cells. The width of the sorus is given at the epidermal level in all the species studied (*table 2*) to give an indication of the amount of distention of the

stoma occurring in the various soral formations. The substomal and the superstomal measurements have also been included to give an idea of the size of the sorus.

3. *Superstomal "B" type: H. jasmini* K. & R. is chosen as representing this type. The type is characteristic of two species occurring on the Oleaceae and a few species on the Apocynaceae, like *H. strophanthi* Racib. In this type the hyphae of the rust rarely traverse extensively below the epidermis. So far as the sections indicate, they arise near the palisade layer of the comparatively thick leaves and in the vicinity of vascular bundles. The haustoria are formed directly in the sieve tubes or in the nearby host cells. These haustoria are much-branched and fill the cells completely. They measure up to 10 to 12 μ in diameter and are as thick as the host cell. There is also a considerable enlargement of the cell due to the haustorium. From this a single hypha grows toward the substomal space and there develops a large bulbous structure which is dense internally and stains deeply. From this bulbous base in the substomal region there arises either a bundle of vertical hyphae which bear the spores at their tips or specialized basal cells bearing sterigmata (FIGS. 2, 5) cutting off the spores. Some slight deviation from *H. jasmini* was noticed in other species included in this type. For example, in *H. strophanthi* a limited number of hyphae, usually two to five, form the bulbous base from which the sporogenous cells arise. A similar development has been illustrated by Maublanc and Roger (**8**) in *H. coffeicola* Moubl. and Roger. This character along with the different symptoms and spore shape is said to separate this species from *H. vastatrix* also occurring on coffee. The guard cells are undisturbed and the width of the sorus is thus limited by them.

DISCUSSION

Although three general types of sori have been here described for the genus *Hemileia,* there are a few species whose sori cause varying degrees of disruption of the guard cells and the adjacent epidermal cells. *H. jahnii* and *H. paveticola* are such species. Sporulation is strictly superficial to the host but the sporogenous cells form a basal column rupturing the epidermis. Such intermediate species may well be considered as connecting links be-

tween the strictly superstomal and the truly subepidermal species. The fact that both types of sori are found in the three principal host families of the genus (Rubiaceae, 16 spp., Apocynaceae, 8 or 9 spp., and Asclepiadaceae, 5 spp.) containing nearly 75 per cent of all the species, suggests their independent development in each host family. It appears possible that the superstomal sorus is more advanced than the subepidermal since it causes less disruption of the host and thus may increase its own chances of survival.

Considerable variability also exists even within species in the degrees of specialization of the sporogenous basal cells. In both the subepidermal and the superstomal sori they may be highly developed, forming many sterigmata and several successive crops of spores, or the spores may be borne singly at the apices of hyphal pedicels. Within species both pedicellate cells and specialized sporogenous basal cells may occur. For instance, in *H. chlorocodonis,* in the same section the two types were seen in adjacent sori. It is possible that the basal cells are formed as the sorus ages and it may very well be a method for producing a continuous crop of spores over a good length of time, by a typically small sorus like that of *Hemileia.* These facts lend additional support to the contention of Thirumalachar and Cummins (**12**), that the sporogenous basal cells are not of particular taxonomic significance.

The superstomal sporulating habit of the genus suggests a relationship of the genus with *Cystopsora* and *Gerwasia. Gerwasia* as a genus is a dubious item since no one since the author has ever seen the authentic material. The superstomal sporulating character is one of the few distinguishing features of *Hemileia.* The existence of the subepidermal type of sorus in a considerable number of species necessitates a revision of our concept of the genus. The peculiar type of sorus found in *H. jasmini* and *H. strophanthi,* especially the substomal swelling, recalls the type of sorus found in *Desmella.* Whether this morphological similarity has any significance is an open question. Equally suggestive is the morphological similarity of the basal cells found in *Scopella cryptostegiae* Cumm. and in *H. fadogiae* Syd. (FIG. 6). As a matter of fact, if the concept of the genus *Hemileia* is broadened to include the subepidermal sorus with single-celled pedicellate teliospores, then the only point of difference between it and *Scopella* would be the

shape of the urediospores and the teliospores. All that is suggested here is that more solid bases for generic distinction are necessary than the presence or absence of specialized basal cells or the differing shapes of spores. The relationships and status of genera can be definitely settled only after a knowledge of the primary stages, because the spermagonia, so far as known, are the most conservative of the sori. Without implying that the secondary sori are unimportant it may prove true that the primary sori may be trurer indices of phylogenetic relationships than either uredia or telia.

It is interesting that the teliospores in the subepidermal species of *Hemileia* are only doubtfully known, measurements being given for only one of them [*H. holstii* Syd., 14–21 × 11–16 μ (**11**)]. The discovery of the existence of the subepidermal uredia leaves us a choice between subdividing the genus into subgenera or erecting new genera. With pycnia and aecia unknown for any species and with telia unknown or doubtful in several of them, it would not be desirable to segregate the subepidermal species by more than subgeneric rank. Similar variation in sori is known to occur in *Mainsia* Jackson (**5**) and *Prospodium* Arth. (**1**). In the latter genus Cummins (**3**) employed the variant morphology as the basis for subgenera.

All the species of *Hemileia* which the author has observed in nature cause a severe defoliation of the host. As a result of the examination of sections made during this study it was noted, especially where the mycelium is scanty, that it formed basal, branched, robust haustoria in the bundle sheath from which the sorus feeder hyphae arose (*H. jasmini, Hemileia* sp. on *Alafia clusioides, H. deightonii,* and others). It is possible that this method of parasitism by which the parasite directly taps the phloem of the host may contribute to the severe defoliation so often resulting from infection with *Hemileia* sp., especially noted in the rust-infected coffee.

SUMMARY

1. A comparative study of the morphology of the sori in 32 species of *Hemileia* is presented. It is suggested that on the basis of soral morphology and position, the genus may be divided into

subgenera. It is indicated that some named species may really be physiologic races.

2. Pycnia and aecia are unknown. Negative results were obtained with cross inoculations of various unconnected aecia and with reciprocal inoculations using basidiospores from germinating teliospores of *H. vastatrix*.

3. There are three types of sori found in the genus, the subepidermal, the superstomal "A," and the superstomal "B." The superstomal "A" type, with eighteen species, predominates. There are a few intermediate types.

4. The importance of primary sori in the classification of rusts is stressed and support is given to the view that basal cells and teliospore variations are not sure bases for generic distinction.

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EFFECT OF NUTRITION ON THE COLONY CHARACTERISTICS AND MACROCONIDIAL FORMATION OF MICROSPORUM AUDOUINI [1]

ELIZABETH L. HAZEN

(WITH 4 FIGURES)

Of the three recognized microspora (*Microsporum audouini, Microsporum canis, Microsporum gypseum*) only *Microsporum audouini* presents a problem in specific identification. Growth on the commonly used Sabouraud's dextrose, maltose, or honey agars may be sparse and macroconidia scarce or absent, whereas *Microsporum canis* and *Microsporum gypseum* grow luxuriantly and form macroconidia abundantly on these media. Conant (**3**) discovered that *Microsporum audouini* grows feebly and produces no macroconidia on sterilized rice grains—unlike the other two species of *Microsporum*. Benedek (**2**) found that *Microsporum audouini* grows well and produces macroconidia on polished rice grains inoculated with *Bacillus weidmaniensis*. Georg [2] demonstrated that sterile filtrates of broth cultures of *Bacillus weidmaniensis* contained thiamine and inositol but no pyridoxine. Arêa-Leão and Cury (**1**), in studying the vitamin requirements of pathogenic fungi, observed that a strain of *Microsporum audouini* which had been maintained for eight years on artificial media required nicotinic acid for growth. The author (**5**) showed that honey agar supports moderate vegetative growth of *Microsporum audouini*, but few macroconidia are produced on this medium. In many strains none can be found. Additions of yeast extract to the honey medium caused marked increase in vegetative growth and the production of more numerous macroconidia. Additions of pyridoxine

[1] This work was done in the Department of Dermatology, College of Physicians and Surgeons, Columbia University, in association with Dr. Rhoda Benham, to whom I wish to express thanks for advice and suggestions throughout the study.

[2] L. Georg. Personal communication.

(5 μg./ml. of medium) caused no increase in vegetative growth, but did induce some increase in macroconidia. Additions of thiamine, or thiamine and pyridoxine caused no increase in vegetative growth and thiamine caused no increase in spore formation.

This investigation has been continued in an attempt to clarify the nature and effect of the growth-promoting factors involved in the maximal development of *Microsporum audouini.* A variety of nutrients was tested by incorporating them in rice infusion agar and chemically defined agar media. The effect of these nutrients upon both the macroscopic growth and macroconidia formation of this fungus was studied. The results of these studies are reported.

MATERIALS AND METHODS

The two basal agar media and the rice infusion agar were prepared as follows:

Rice Infusion Agar

A—Polished rice grains ..... 100 gms.
Distilled water ..... 1000 ml.
Mix and boil 30 minutes, filter through cheesecloth and make up to 500 ml.
B—Difco Bacto agar ..... 20 gms.
Distilled water ..... 500 ml.
Mix and heat until agar is melted.
Combine A and B and sterilize at 20 lbs. pressure for 20 minutes.

Basal Medium A

Asparagine (recrystallized)	2.0 gms.
Dextrose, C.P.	50.0 gms.
$MgSO_4 7H_2O$ (C.P.)	0.1 gm.
Purified agar (**4**)	1.5 gms.
Sorensen's phosphate buffer solution (*M*/15 KH_2PO_4 and Na_2HPO_4 at pH 7.0)	100 ml.
Distilled water	1000 ml.

Sterilize at 15 lbs. pressure for 15 or 20 minutes.

Basal Medium B

Same composition as Medium A, with the exception that the asparagine was replaced by 2 gms. NH_4Cl (C.P.).

Pure cultures of *Microsporum audouini,* isolated from cases of tinea capitis, were used. All isolations were fairly recent except Nos. 7 and 8 which had been employed in the earlier study (**5**). The cultures were maintained on Sabouraud's honey agar; growth was velvety and sparse and macroconidia were not demonstrated.

Fresh sterile solutions of the B complex vitamins in distilled water, singly, or in different combinations, were added in the following amounts per ml. of medium: thiamine 0.1 μg., pyridoxine 0.1 μg., inositol 5.0 μg., biotin 0.0005 μg., nicotinomide 5.0 μg., pantothenic acid 5.0 μg., PAB 5.0 μg., and riboflavin 0.05 ml. of a saturated solution. Five per cent solution of the yeast extract (a dehydrated Difco product) is distilled water was sterilized by filtration through a Seitz filter and added to the media in a concentration of 5 mgs. per ml. Dextrose and asparagine were added to rice infusion agar in concentrations of 50 and 2 mgs. per ml., respectively.

The inocula for the experimental media were taken from honey agar slants and washed thoroughly several times in sterile distilled water, or from at least a second transfer on basal media. Pinpoint size inocula were placed in the center of Petri dishes (65 or 100 mm. in diameter) containing, respectively, 15 and 25 mls. The plates remained at room temperature for eight weeks or longer. The cultures were inoculated in duplicate and the character of growth and diameter of colonies were recorded usually at weekly intervals for eight or ten weeks. When the cultures were about three weeks old, and thereafter at weekly intervals, as far as possible, up to ten weeks, film preparations were made and examined for macroconidia, as previously described (**5**). The number of macroconidia in a culture was roughly estimated from the total number found in the film preparations during the entire period of examination.

Thirteen cultures were studied: five on rice infusion agar, and on this medium plus dextrose, asparagine, yeast extract, added singly, and in combination, and B vitamins; seven on the basal medium A and on this medium plus yeast extract, and B vitamins added, separately, and in combination; eleven on the basal medium B and on this medium plus B vitamins added, singly, and combined.

The effect upon growth was evaluated on the basis of macroscopic growth, as indicated by colony diameter, character of the mycelium, relative amount of aerial growth, and macroconidial production.

TABLE I

EFFECT UPON GROWTH* AND MACROCONIDIAL FORMATION† OF *M. audouini* ON RICE INFUSION AGAR WITH AND WITHOUT VARIOUS NUTRIENTS

Culture No.	Rice Infusion Agar		Rice Infusion Agar plus Dextrose 50 mg./ml.		Rice Infusion Agar plus Asparagine 2 mg./ml.		Rice Infusion Agar plus Dextrose and Asparagine (same as single amts.)		Rice Infusion Agar plus Yeast Extract 5 mg./ml.		Rice Infusion Agar plus Dextrose, Asparagine, Yeast Extract (same as single amts.)		Rice Infusion Agar plus B Vitamins	
	Growth 4–6 wks.	Macroconidia 4–8 wks.	Growth 4–6 wks.	Macroconidia 4–8 wks.	Growth 4–6 wks.	Macroconidia 4–8 wks.	Growth 4–6 wks.	Macroconidia 4–8 wks.	Growth 4–6 wks.	Macroconidia 4–8 wks.	Growth 4–6 wks.	Macroconidia 4–8 wks.	Growth 4–6 wks.	Macroconidia 4–8 wks.
7	±		+	0	+	0	++	0	+++	0	++++	++++	±	
8	±		+	0	+	0	++	0	+++	0	++++	+	±	
21	±		+	0	+	0	++	0	+++	0	++++	+	±	
22	±	0					++	0	+++	0	++++	++	±	
23	±		+	0	+	0	++	0	+++	0	++++	−	±	

* ± = Feeble submerged growth.
\+ = Moderately heavy subsurface growth with scanty aerial hyphae.
++ = Heavy subsurface growth with moderate aerial mycelium.
+++ = Colony 4–6 cm. in diameter, heavy subsurface growth with velvety mycelium.
++++ = Colony 6–8 cm. in diameter, heavy velvety to fluffy or finely powdered mycelium.
† No spores = 0; 1–5 spores = +; 6–10 spores = ++; more than 10 spores = ++++.

EXPERIMENTAL

The results of these studies are summarized in tables I–IV.

TABLE I—*Rice Infusion Agar Medium*

1. Growth of the five strains on the rice infusion medium was rapid and completely submerged, and was so thin as to be discernible only by strong transmitted light (FIG. 1a).

2. With addition of dextrose to the medium, the four cultures studied developed a fairly heavy, rapidly spreading, submerged mycelium with a moderate number of aerial hyphae; with addition of asparagine growth was less rapid but of greater density than on the dextrose medium (FIGS. 1b and 1c). The two materials combined increased further the aerial growth (FIG. 1d).

3. With the addition of yeast extract alone, all of the strains showed heavy subsurface growth covered with thick velvety mycelium (FIG. 1e).

4. Asparagine, dextrose and yeast extract combined resulted in production of large and more typical colonies with heavy velvety to fluffy or finely powdery mycelium (FIG. 1f).

5. Addition of the following mixture of vitamins—thiamine, pyridoxine, inositol, nicotinomide, pantothenic acid, p-aminobenzoic acid, biotin, and riboflavin—did not influence mycelial growth.

6. In four strains macrospores were found only on the medium reinforced with yeast and asparagine and dextrose. Even there, except for No. 7 which formed these spores in large numbers, they were so scanty that no definite conclusion could be drawn.

These results suggest that for maximal development of *Microsporum audouini* a suitable source of carbon and nitrogen and some unknown factor or factors contained in yeast extract are essential.

TABLE II—*Basal Medium A*

1. Six of seven strains showed fair mycelial development on this basal medium. The seventh showed only feeble submerged growth without aerial hyphae.

2. Three of seven strains produced macroconidia on this medium, No. 7 forming them in large numbers.

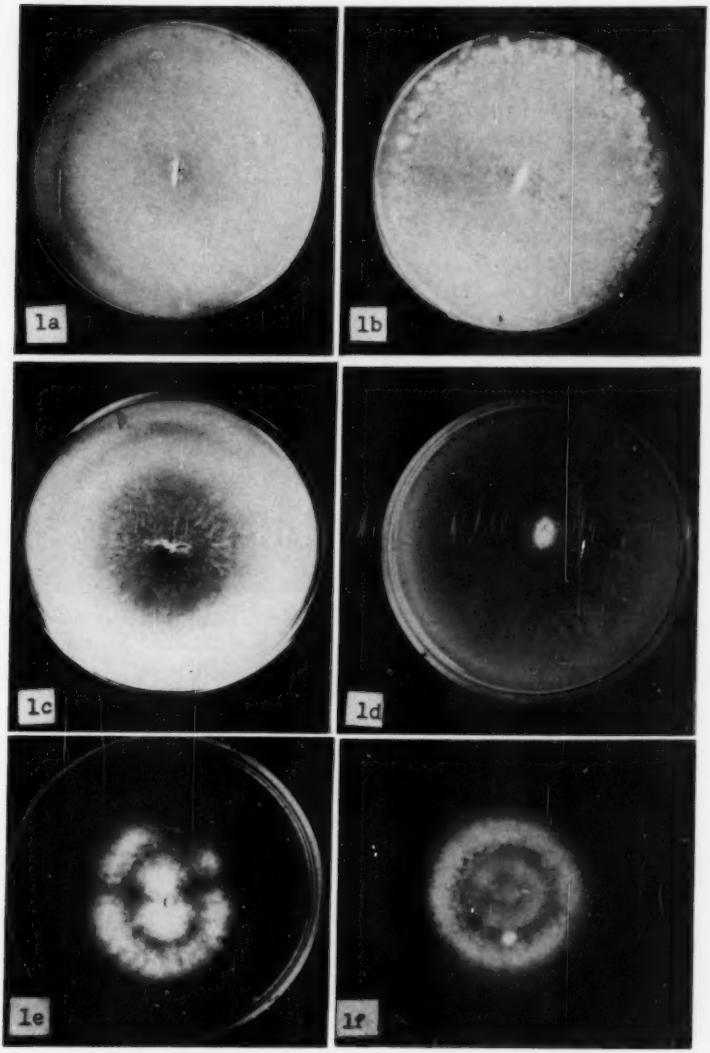


FIG. 1a. *Microsporum audouini.* Strain No. 22, rice infusion agar medium, 4 weeks. b. Strain No. 22, rice infusion agar medium, plus dextrose, 4 weeks. c. Strain No. 22, rice infusion agar medium, plus asparagine, 4 weeks. d. Strain No. 22, rice infusion agar medium, plus dextrose and asparagine, 4 weeks. e. Strain No. 22, rice infusion agar medium, plus yeast extract, 4 weeks. f. Strain No. 23, rice infusion agar medium, plus dextrose, asparagine, and yeast extract, 4 weeks.

TABLE II

COLONY DIAMETER (IN MM.) AND ESTIMATE* OF NUMBER OF MACROCONIDIA PRODUCED BY *M. audouini* ON ASPARAGINE-DEXTROSE MEDIUM WITH AND WITHOUT B VITAMINS

Culture No.	Basal Medium A (Asparagine-Dextrose)		Basal Medium B plus Yeast Extract (5 mg. per ml.)		Basal Medium A plus Thiamine (0.1 μg. per ml.)		Basal Medium A plus Pyridoxine (0.1 μg. per ml.)		Basal Medium A plus Inositol (5 μg. per ml.)		Basal Medium A plus Biotin (0.0005 μg. per ml.)		Basal Medium A plus Thiamine Pyridoxine Biotin Inositol (same as single amount)	
	Diam. 1 month mm.	Macroconidia within 6 wks.	Diam. 1 month mm.	Macroconidia within 6 wks.	Diam. 1 month mm.	Macroconidia within 6 wks.	Diam. 1 month mm.	Macroconidia within 6 wks.	Diam. 1 month mm.	Macroconidia within 6 wks.	Diam. 1 month mm.	Macroconidia within 6 wks.	Diam. 1 month mm.	Macroconidia within 6 wks.
7	30	++++	65	++++	28	+	30	++++	27	++	32	++++	25	++++
8	26	+	65	++++	37	+	35	−	40	−	30	+	34	+
19	ss†	+	65	+	ss	−	ss	−	ss	−	ss	+	ss	−
20	23	−	65	+	20	−	20	−	19	+	30	−	20	+
21	15	−	58	−										
22	25	−	60	+										
23	29	−	58	−										

† ss = Subsurface growth.
* No spores = −; 1–5 spores = +; 6–10 spores = ++; more than 10 spores = ++++.

3. Yeast extract increased mycelial growth in all strains. Even No. 19, which showed minimal growth on the basal medium, formed a large typical colony when yeast extract was added.

4. Thiamine, pyridoxine, inositol, and biotin, alone or combined, failed to show this effect.

These results suggest that none of the four vitamins, at least in the amounts tested, is sufficient to permit vigorous mycelial or

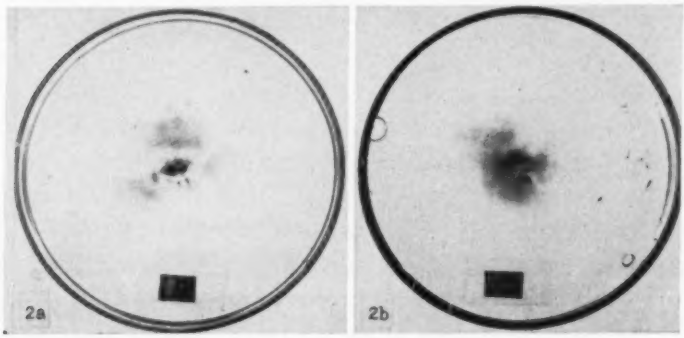


FIG. 2a. *Microsporum audouini.* Strain No. 29, ammonium chloride-dextrose agar medium, 4 weeks, photographed by transmitted light. b. Strain No. 29, ammonium chloride medium plus thiamine, pyridoxine, biotin, inositol, 4 weeks, photographed by transmitted light.

macrospore development of this fungus. Asparagine is an adequate source of nitrogen. Yeast extract contains some substance or a combination of substances which stimulates mycelial growth.

TABLE III—*Basal Medium B*

1. Eight of eleven strains showed only feeble, submerged growth with few aerial hyphae on this medium (FIG. 2a); three developed velvety to fluffy aerial hyphae.

2. Additions of thiamine, peridoxine, biotin, and inositol, in three combinations, produced no increase in mycelial growth (FIG. 2b).

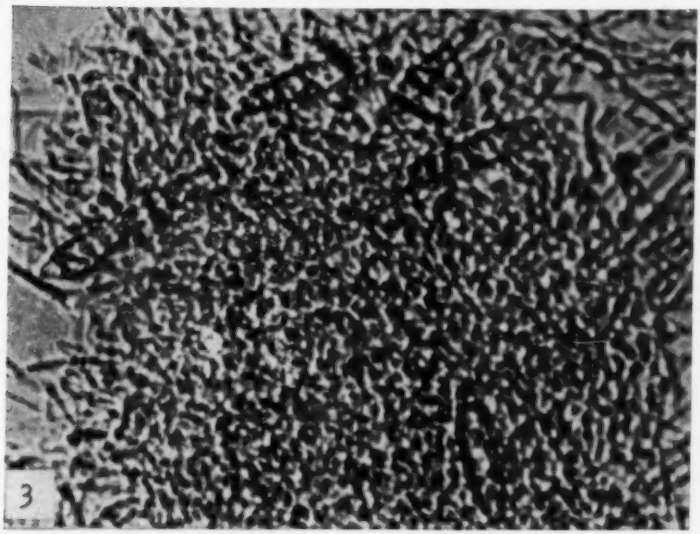
3. Six of eleven strains produced a few, and a seventh numerous macroconidia on this basal medium. The spores were typical in morphology (FIGS. 3, 4). There was slight increase in macrospore

TABLE III

COLONY DIAMETER (IN MM.) AND ESTIMATE* OF NUMBER OF MACROCONIDIA PRODUCED BY *M. audouini* ON AMMONIUM CHLORIDE-DEXTROSE MEDIUM WITH AND WITHOUT B VITAMINS

Culture No.	Basal Medium B (NH_4Cl-Dextrose)		Basal Medium B plus Thiamine 0.1 μg. per ml. Pyridoxine 0.1 μg. per ml. Biotin 0.0005 μg. per ml.		Basal Medium B plus Thiamine 0.1 μg. per ml. Pyridoxine 0.1 μg. per ml. Inositol 5.0 μg. per ml.		Basal Medium B plus Thiamine 0.1 μg. per ml. Pyridoxine 0.1 μg. per ml. Biotin 0.0005 μg. per ml. Inositol 5.0 μg. per ml.	
	Diam. 5 wks. mm.	Macroconidia within 8 wks.	Diam. 5 wks. mm.	Macroconidia within 8 wks.	Diam. 5 wks. mm.	Macroconidia within 8 wks.	Diam. 5 wks. mm.	Macroconidia within 8 wks.
7**	18.5	−	24	+	20	−	25	−
8**	ss†	−	ss	−	ss	+	ss	+
21	30	−	40	−	35	−	40	−
22	14	+	22	−	18	−	17	−
23	ss	+	ss	+	ss	++	ss	++
24	ss	+	ss	−	ss	++	ss	−
25	ss	+	ss	+	ss	+	ss	+
26	ss	−	ss	−	ss	+		
27	ss	+	ss	−	ss	++	ss	+
28	ss	+	ss	−	ss	+	ss	++
29	ss	++++	ss	++++	ss	+++	ss	++++

† ss = scanty, colorless mycelium, chiefly subsurface.
* No spores = −; 1–5 spores = +; 6–10 spores = ++; more than 10 spores = ++++.
** Strains had been maintained on artificial media for almost three years.



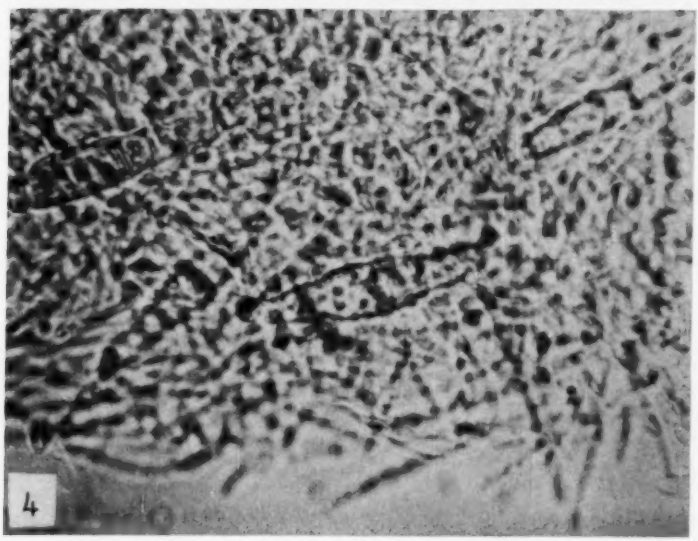


FIG. 3. Macroconidia of *Microsporum audouini* formed on ammonium chloride-dextrose medium.

FIG. 4. Same, enlarged.

TABLE IV

MACROCONIDIAL FORMATION OF *M. audouini* ON AMMONIUM CHLORIDE MEDIUM WITH AND WITHOUT B VITAMINS

Culture No.	Estimate of Number of Macroconidia in Culture*							
	Basal Medium B (NH_4Cl-Dextrose)	Basal Medium B plus Thiamine chloride 0.1 μg./ml. Inositol 5 μg./ml.	Basal Medium B plus Pyridoxine 0.1 μg./ml. Inositol 5 μg./ml.	Basal Medium B plus Thiamine Pyridoxine (same as single amts.)	Basal Medium B plus Thiamine Pyridoxine (same as single amts.) Inositol 5 μg./ml.	Basal Medium B plus Inositol 2.5 μg./ml.	Basal Medium B plus Inositol 5 μg./ml.	Basal Medium B plus Inositol 10 μg./ml.
23	−	−	+	−	−	−	+	−
25	+	++	++	++	++	−	++	+
27	+	−	+	−	++	+	+	++
28	+	+	+	++++	+	+	−	++

* No spores = −; 1–5 spores = +; 6–10 spores = ++; more than 10 spores = ++++.

formation in some instances when inositol had been added but the results were irregular and inconclusive.

Since there was a possibility that growth on the ammonium chloride medium might have been due to transfer of sufficient nitrogen with the inoculum and not to utilization of ammonium as the source of nitrogen, Basal Medium B with and without NH_4Cl was tested for ability to support growth of carefully washed fragments of mycelium. Three older strains (Nos. 22, 25, 29), and three freshly isolated strains (Nos. 30, 31, 32), were employed. Growth of all six strains occurred on the medium with NH_4Cl and no growth on the medium without NH_4Cl. Growth of these cultures has also been maintained for five or more transfers to the medium with NH_4Cl. It would therefore appear that *Microsporum audouini* can utilize the ammonium as a source of nitrogen and that the growth of strains (TABLE II) was not due to nitrogen transferred with the inoculum.

TABLE IV—*Basal Medium B*

For additional evidence that inositol influenced spore formation, four strains which had previously produced few macroconidia on this basal medium were tested on this medium with the addition of various combinations of thiamine, pyridoxine, and inositol, and inositol, alone, in graded concentrations. The results were again irregular and inconclusive. The abundance of macrospores was determined more by some property of the strains than by the medium used, *e.g.*, strain No. 7 produced numerous macroconidia on Basal Medium A, and No. 29 on Basal Medium B, without yeast or vitamin supplements, while the other strains rarely showed more than an occasional macrospore. No factor was found which induced abundant macrospore formation in all strains.

SUMMARY AND CONCLUSIONS

Microsporum audouini produces a feeble submerged mycelium on rice infusion agar. Addition of glucose and asparagine, combined or separately, increased mycelial development with formation of aerial hyphae. Addition of yeast extract promoted to a marked degree the vegetative growth of the fungus. The growth-promoting factors involved here would not seem to be thiamine, pyridoxine,

biotin, inositol, nicotinomide, riboflavin, and p-aminobenzoic acid, since a mixture of these vitamins added to the medium did not benefit growth. Maximal development of the fungus occurs when glucose, asparagine, and yeast extract are added in combination.

Microsporum audouini can utilize ammonium as a source of nitrogen for limited subsurface growth of freshly isolated strains or for development of aerial mycelium of older stock strains.

Mycelial growth is increased by substitution of asparagine for ammonium chloride and still further by addition of yeast extract.

A source of nitrogen suitable for the particular strain seems necessary for the formation of macrospores. There are indications that inositol and some other factor in yeast extract favor their production.

The abundance of macrospores was determined, apparently, more by some property of the strains than by the medium used. For example, strain No. 7 produced numerous macroconidia on asparagine-dextrose medium (Basal Medium A) and No. 29 on ammonium chloride medium (Basal Medium B) without yeast extract or vitamin supplements, while the other strains rarely showed more than an occasional macrospore. No factor was found which induced abundant macrospore formation in all strains.

Further nutritional studies of this species are in progress, particularly with reference to its nitrogen requirements.

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THE RELATION OF NUTRITION TO THE GROWTH AND MORPHOLOGY OF TRICHOPHYTON VIOLACEUM. I. THE VITAMIN AND AMINO ACID REQUIREMENTS OF T. VIOLACEUM [1]

LUCILLE K. GEORG

(WITH 3 FIGURES)

This study was undertaken to determine the optimum conditions for growth and spore production of *Trichophyton violaceum*. This organism has been reported to be the commonest cause of ringworm of the scalp in many of the Mediterranean countries and has been isolated frequently in Bulgaria, Russia, and China, but occurs only rarely in the United States.

T. violaceum grows very slowly on Sabouraud's dextrose agar, forming a small, moist, glabrous, white to greyish, heaped and verrucose colony that later develops a lavender to deep purple pigment. Microscopic examination reveals a mass of poorly formed mycelium. The only other structures usually seen are chlamydospores. The cultures tend to die unless transferred frequently to fresh media. Stock cultures undergo changes of several types: 1. the development of lightly pigmented or colorless sectors of moist, heaped, glabrous growth; 2. development of a greyish-white powder over the surface of colony; 3. the development of flat spreading sectors covered with a short, fine white aerial growth or "down" which may bear a few delicate microconidia; and, finally, 4. either the production of a grey, heaped colony of rubbery texture which may bear spine-like outgrowths of long twisted aerial mycelium or 5. the development of rapidly-growing white, woolly, sterile mycelium which may cover the entire colony. Figure 1 shows variations of the colonial form.

[1] From the Department of Dermatology, College of Physicians and Surgeons, Columbia University, New York City.

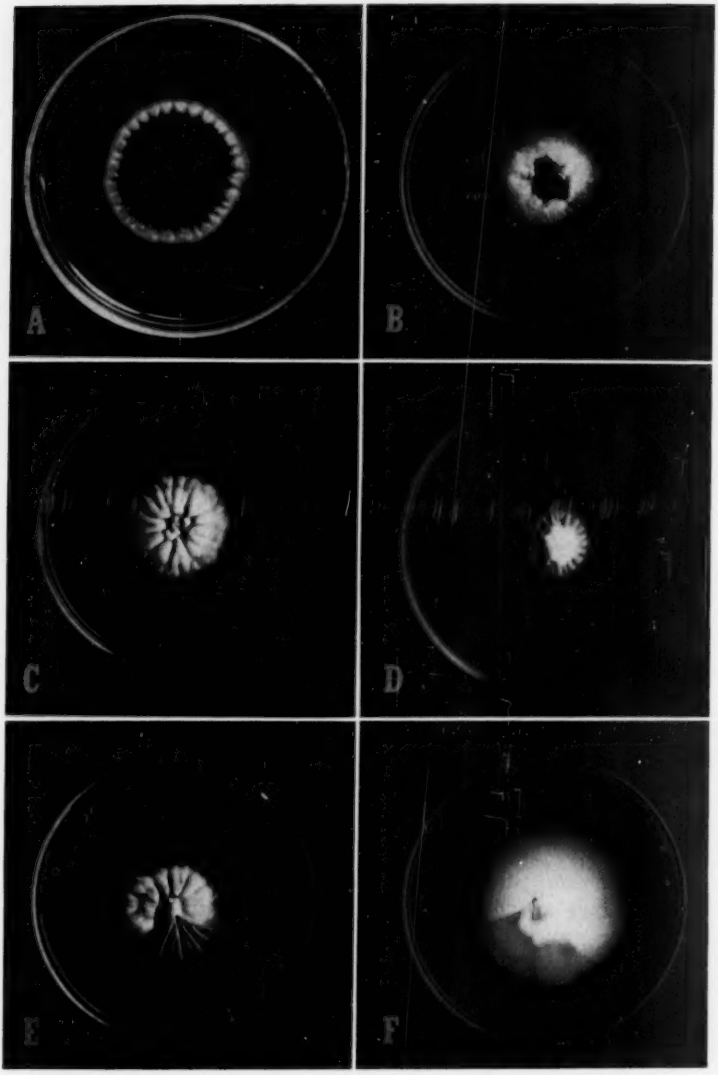

FIG. 1. Variations in the colonial form of *T. violaceum* on Sabouraud's dextrose agar (Difco).

T. violaceum has been placed in the group of "faviform trichophytons" along with *T. Schoenleini* and *T. faviforme* because of its superficial similarity to the slow-growing, glabrous, non-sporulating colonies of these fungi. Langeron and Milochévitch (**5**) were the first to demonstrate, however, that many of the strains in this group could be stimulated by natural carbohydrate media. On such media, which consisted of whole grains of wheat, barley, corn, and oats, these fungi produced a rapidly growing, powdery to downy colony in which regular, well-formed mycelium and, in some instances, microconidia were demonstrated.

The stimulation of growth and spore production on natural media suggested that the latter were rich in growth factors, such as vitamins and amino acids required by these organisms for their normal development. Burkholder and Moyer (**4**) reported that a strain of *T. violaceum* which they studied was deficient for thiamine and its growth was stimulated by liver extract and certain peptones. Arêa-Leão and Cury (**1**), in a survey of the vitamin requirements of pathogenic fungi, reported that a strain of *T. violaceum* (No. *1254*) would not grow in a basal mineral medium containing dextrose and asparagine in the presence of any or all of 7 vitamins tested, including thiamine. However, they obtained good growth when yeast extract was added to the medium.

MATERIALS AND METHODS

Eleven strains of *T. violaceum* were studied on synthetic, chemically defined media in order to determine their vitamin and amino acid requirements. Two were freshly isolated strains, seven had been in stock culture for several months to several years, and two were obtained from the Central Bureau for Fungus Cultures, Baarn, Netherlands (*T. violaceum* strain Leontieff and *T. glabrum* strain Engelhardt). This latter strain was included in the study as *T. glabrum* has been considered to be a non-pigmented variety of *T. violaceum*. These cultures comprised all the variations in colony form described above, and ten of the eleven showed the characteristically impoverished microscopic morphology on Sabouraud's dextrose agar. One strain, *T. violaceum* (Leontieff), obtained from The Netherlands Collection was unusual. It developed more rapidly than the others, covering the medium with a white

fluffy growth. Grossly, it appeared to be pleomorphic, but on microscopic examination it was found that the fluffy, white aerial mycelium was not sterile but bore many tiny microconidia. As will be pointed out later, this culture (No. *365*) proved to be completely different in its nutritional requirements from the other ten strains studied, which perhaps accounts for the difference in morphology.

A basal vitamin-free medium was prepared as follows: 50 g. dextrose and 0.5 g. $MgSO_4$ were dissolved in a liter of distilled water buffered to pH 7 with Sorensen's phosphate mixture (KH_2PO_4 and Na_2HPO_4). Various nitrogen sources were added in 0.2 per cent amounts: NH_4Cl, $NaNO_3$, NH_4NO_3, or vitamin-free, acid-digested casein. (All of the chemicals were of C. P. quality. The acid-digested casein was obtained from General Biochemicals Co.) For solid media, 1.5 per cent purified agar, prepared according to the method of Robbins (**6**), was added. The media were autoclaved for 15 min. at 120° C. All vitamins and amino acids were prepared in water solution, sterilized by filtration, and added to the melted, partially cooled, basal agar.

RESULTS

Utilization of nitrogen sources: The ten typical strains could be maintained on a vitamin-free, dextrose agar with either inorganic or organic nitrogen. Availability of nitrogen was in the following order: acid-digested casein > NH_4NO_3 > NH_4Cl > $NaNO_3$. Development on such media, however, was very restricted and consisted of a thin, submerged growth which spread 5 to 10 mm. from the point of inoculation. The thin, poorly formed mycelium remained viable for 6 to 8 weeks and could be subcultured indefinitely on similar media. Increasing the amount of inorganic nitrogen compound or casein in the medium did not increase the amount of growth. This small amount of submerged growth was recorded as +, and the growth on 0.2 per cent NH_4NO_3 basal agar was used as the source of inoculum for studies with vitamin-enriched and amino acid-enriched media.

In contrast to these ten strains, strain No. *365* did not grow on the inorganic nitrogen media. However, this strain grew well on

the casein agar, and the amount of growth was roughly proportional to the amount of casein present.

Vitamin requirements: All strains were tested on the vitamin-free, dextrose basal agar with 0.2 per cent NH_4NO_3 or 0.2 per cent acid-digested casein for possible stimulation by the following vitamins, singly or in combination: thiamine, biotin, nicotinic acid, nicotinic amide, pyridoxine, riboflavin, i-inositol, choline, calcium pantothenate, para-aminobenzoic acid, and folic acid. Single vitamins were added in amounts of 0.1 mg. to 5 ml. basal agar, except biotin of which 0.05 mg. was added.

TABLE I

TITRATIONS OF *T. violaceum* (STRAIN *365a*) WITH VARIOUS GROWTH FACTORS

Estimates of amount of growth* produced in test tubes containing 5 ml. NH_4NO_3 broth plus various increments of the growth factors.

A. Titration with para-amino-benzoic acid		B. Titration with thiamine		C. Titration with oxalacetate	
Gammas of PABA per tube	Growth	Gammas of thiamine per tube	Growth	Gammas of oxalacetate per tube	Growth
None	±	None	±	None	±
0.000–39.0	±	0.0000–0.0005	±	0.1–39.0	±
78.0–312.5	1+	0.002–0.003	1+	78.0–156.0	1+
625	2+	0.0075–0.015	2+	312.5–5000.0	2+
1,250–2,500	3+	0.03–1.0	3+		
		1.95–500.0	4+		
m.e.c.** = 78.0 μg.		m.e.c.** = 0.002 μg.		m.e.c.** = 78.0 μg.	

* Growth after incubation for 14 days at 25° C.
± Small ball of growth in bottom of tube.
1+ Growth filling approximately ¼ of broth.
2+ Growth filling approximately ½ of broth.
3+ Growth filling approximately ¾ of broth.
4+ Growth completely filling broth.
** m.e.c. = minimum effective concentration.

The growth of ten of the eleven strains (all except No. *365*) was greatly stimulated by the addition of thiamine to either the NH_4NO_3 or acid-digested casein basal agar. The only other vitamin which showed a stimulatory effect was para-aminobenzoic acid. The effect of this vitamin was slight, but definite, for all ten strains. It was not apparent unless comparatively large quantities, such as 78 or more micrograms, were added to 5 ml. of NH_4NO_3

basal broth. Growth, after incubation for 14 days at 25° C., was recorded as 0 to 4+ (TABLE I). To determine whether this effect was due to possible contamination with thiamine, the PABA solutions were tested with washed spores of *Phycomyces Blakesleeanus* which germinate in the presence of minute traces of thiamine (3). No germination occurred.

The minimum effective concentration of thiamine was determined by adding diminishing amounts of this vitamin to a series of tubes each containing 5 ml. NH_4NO_3 broth and inoculating with small fragments of the culture grown on NH_4NO_3 basal agar. Although a small amount of growth occurred in all tubes, definite stimulation by the thiamine was observed in all tubes down to one containing 0.002 μg. in 5 ml. NH_4NO_3 broth. A gradual increase in amount of growth was observed up to a dilution containing 0.5 μg. Larger quantities of thiamine had no further effect. No inhibitory effect could be seen with large doses of thiamine (up to 0.5 mg. in 5 ml. NH_4NO_3 broth) (TABLE I). In addition it was found that only the pyrimidine portion of the thiamine molecule was active, and molecular thiamine could be replaced by an equi-molar amount of 2-ethoxy-methyl-6 amino pyrimidine. Oxalacetate was found to substitute partially for thiamine. The strain classified as *T. glabrum* reacted similarly to the other 9 strains tested (FIG. 2A, B).

With the addition of adequate dosages of thiamine (1.0 μg. or more) to 10 ml. of NH_4NO_3 basal agar, rapid growth of all ten strains occurred. In many strains, downy to fluffy colonies were obtained. Increasing the amount of NH_4NO_3 in the medium did not further increase growth. The amount of growth on thiamine-enriched, acid-digested casein agar was similar in amount. However, here it was found that increasing the nitrogen source (acid-digested casein) five-fold greatly increased the amount of the growth. These results indicated that not only were thiamine and to some slight extent para-aminobenzoic acid stimulatory to these strains, but an increase in the amount of organic nitrogen available greatly enhanced the effect of the vitamins.

Strain No. *365* showed no growth on 0.2 per cent NH_4NO_3 agar, nor did any growth occur when large amounts of thiamine, para-aminobenzoic acid, or any combinations of the vitamins were

added. The strain grew well, however, on the 0.2 per cent vitamin-free acid-digested casein agar. It was apparent that this was not a vitamin-deficient strain, but that it was completely deficient for

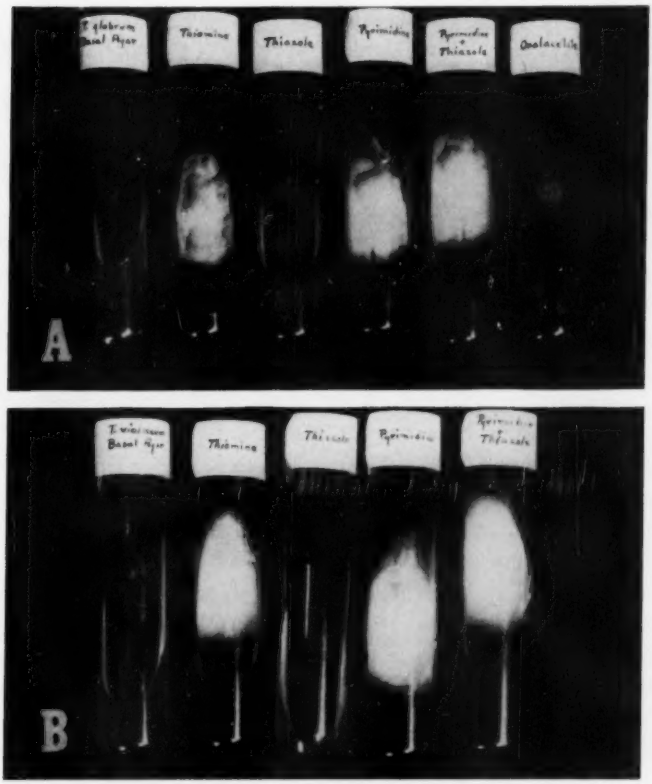

FIG. 2A. *T. glabrum* (Engelhardt), a variant of *T. violaceum*, on basal agar, with the addition of thiamine or its intermediates, and also with oxalacetate. B. *T. violaceum* (No. *365a*) on basal agar, and with the additions of thiamine or its intermediates.

some amino acid or combination of amino acids present in the casein hydrolysate. Increasing the casein content of the medium caused a concomitant increase in growth, and on a one per cent casein agar a rapidly growing, folded colony was obtained.

Amino acid requirements: All strains were tested on NH_4NO_3 basal agar with the addition of the following amino acids, singly and in combination: glycine, dl-alpha alanine, beta alanine, dl-serine, dl-threonine, dl-valine, l-leucine, dl-isoleucine, dl-norleucine, dl-aspartic acid, l-glutamic acid, l-lysine, l-arginine, l-histidine, l-cystine, l-cysteine, dl-methionine, l-proline, l-tryptophane, l-tyrosine, dl-phenylalanine, l-hydroxyproline. These were tested in M/1000 dilution with the exception of cystine which, being highly insoluble, was tested in greater dilution.

Strain No. *365* was found to have a complete deficiency for l-histidine, growth occurring on the NH_4NO_3 basal agar only when this amino acid had been added. The minimum effective concentration of histidine was determined by addition of diminishing amounts of this amino acid to a series of tubes each containing 5 ml. NH_4NO_3 basal broth, and inoculation with small fragments of the culture grown on a minimum casein basal agar. The smallest amount of histidine which would allow growth was 0.0003 mg. in 5 ml. NH_4NO_3 basal broth. Maximum effect was observed in tubes containing 0.01 to 0.08 mg. In dilutions containing 1.5 mg. some toxic effects were observed. Only 1 + growth occurred in a dilution containing 10 mg. histidine (TABLE II).

The maximum effective concentration of histidine, however, allowed only a very slow and meager growth on the NH_4NO_3 basal agar, and it was apparent that addition of other amino acids would be necessary to produce growth comparable to that observed on the 0.2 per cent casein basal agar. The remaining amino acids were added in M/1000 quantities in combination with histidine. It was found that only certain amino acids were utilized. The most effective combinations with histidine are listed in the order of their availability to this strain: glutamic acid + arginine > aspartic acid + arginine > aspartic acid + glutamic acid = lysine + arginine > glutamic acid > aspartic acid > arginine > lysine. Other amino acids which showed some slight stimulatory effect in combination with histidine were alpha alanine, beta alanine, phenylalanine, leucine, valine, glycine, serine, tyrosine, and proline. Of all the amino acids tested, glutamic acid appeared to be the most effective, the combination of glutamic acid and histidine allowing growth comparable to that obtained on 0.2 per cent casein basal

agar. The effective dosages of glutamic acid were determined by serial dilution in tubes containing 5 ml. NH_4NO_3 basal broth and an approximate minimum effective dose of histidine (0.5 mg. per 5 ml.), and inoculation with tiny fragments of the culture grown on slants of a minimum casein basal agar. Doses of glutamic acid

TABLE II

TITRATIONS OF *T. violaceum*, STRAIN 365, WITH HISTIDINE AND WITH HYDROXYPROLINE IN THE PRESENCE OF HISTIDINE

Estimates of amount of growth* produced in test tubes containing 5 ml. NH_4NO_3 broth plus various increments of the amino acids.

Titration with histidine		Titration with hydroxyproline	
Milligrams of histidine per 5 ml. NH_4NO_3 broth	Growth	Milligrams of hydroxyproline per 5 ml. NH_4NO_3 broth containing 10 μg. histidine	Growth
No histidine	0		
0.00006	0	No hydroxyproline	4+
0.0001	±	0.01–0.025	4+
0.0003–0.0006	1+	0.05–0.1	3+
0.001	2+	0.2–0.78	2+
0.002–0.009	3+	1.56	1+
0.019–0.2	4+	3.1–25.0	0
0.4–0.7	3+	No histidine or hydroxyproline	0
1.3–4.9	2+		
9.8–38.2	1+		
m.e.c.** = 0.0003 mg.		m.i.c.*** = 3.1 mg. hydroxyproline	

* Growth after incubation for 14 days at 25° C.
± Small ball of growth in bottom of tube.
1+ Growth filling approximately ¼ of broth.
2+ Growth filling approximately ½ of broth.
3+ Growth filling approximately ¾ of broth.
4+ Growth completely filling broth.
** Minimum effective concentration.
*** Minimum inhibiting concentration.

greater than 500 μg. per 5 ml. NH_4NO_3-histidine basal broth showed a toxic effect, but in higher dilutions good growth occurred up to a dilution containing 0.025 μg. glutamic acid. Above this dilution, growth gradually dropped off and tubes containing 0.001 μg. or less showed no more growth than that produced by histidine alone.

In the presence of histidine, hydroxyproline was found to have a partial inhibitory effect on this strain in a dilution as high as M/1000. Serial dilutions of hydroxyproline were made in tubes containing 5 ml. NH_4NO_3 basal broth and the approximate maximum effective dosage of histidine (10 μg. per 5 ml. broth). Some

inhibition of growth was apparent in tubes containing as little as 0.05 mg. hydroxyproline in 5 ml. NH_4NO_3-histidine broth. Complete inhibition occurred with 3 mg. hydroxyproline (TABLE II). This inhibition could be overcome by adding proline to the medium. Robbins and McVeigh (**8**) have reported a similar inhibition of the growth of *T. mentagrophytes* and other *Trichophytons* by hydroxyproline, which could be overcome by proline. It seems apparent that all of these hydroxyproline-sensitive strains require proline in their metabolism.

The ten thiamine-deficient strains were stimulated by amino acids only in the presence of thiamine. The amino acids which

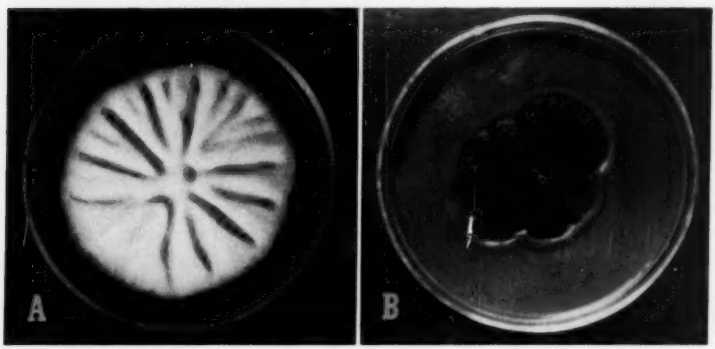

FIG. 3A. *T. violaceum* (No. *365*), a histidine-less strain. B. *T. violaceum* (No. *365a*), a thiamine-less strain derived from a single spore culture of No. *365*.

stimulated growth at a dilution of M/1000 were: glutamic acid, arginine, aspartic acid, histidine, phenylalanine, and proline. No inhibitory effect due to hydroxyproline was observed when the organisms were grown on the NH_4NO_3-thiamine basal broth containing this agent. In the casein-thiamine basal broth, however, a slight inhibitory effect was observed with 15 mg. hydroxyproline per 5 ml. of broth. This corresponded to the findings of Robbins and McVeigh (**8**), who showed that the normal form of *T. mentagrophytes* (the amino acid-requiring form) was much more sensitive to hydroxyproline than the pleomorphic form, which could utilize inorganic nitrogen.

The fact that one of the eleven strains studied (strain No. *365*) was physiologically different from the others, suggested that this might be an unusual mutant form or even a different species. However, studies with a single-spore strain obtained from this culture demonstrated that this strain produced a typical endothrix infection when a spore suspension was inoculated into the shaved, scarified side of a dog. (Several attempts to produce infections in guinea pigs and rabbits failed, or at best produced only erythema and scaling.) The culture recovered from the dog was similar to the white, fluffy strain used as inoculum. On several occasions this pure strain culture gave rise to sectors of glabrous, dark purple growth which was isolated in pure culture characteristic of *T. violaceum* (FIG. 3A, B). The secondary purple growth was apparently a back mutation to the more common form of this species. Upon study, this pigmented mutant (strain No. *365a*) was found not only to have lost its deficiency for histidine, but to have developed a deficiency for thiamine, thus acquiring properties identical to those of the ten other *T. violaceum* strains studied in this series.

DISCUSSION

Study of the vitamin and amino acid requirements of *T. violaceum* indicates that 10 of 11 strains studied, including a non-pigmented strain classified as *T. glabrum*, have in common a requirement for thiamine; or more exactly for the pyrimidine portion of the thiamine molecule. The action of thiamine is quantitative and, within certain limits, an increase in the dosage causes a corresponding increase in growth. When the optimum dosage is reached, further additions are without effect. The fact that the fungus is able to grow slightly and to survive over a series of transplantings on NH_4NO_3 vitamin-free medium suggests that either it is not completely deficient for pyrimidine, i.e., it is able to synthesize a small amount, or it is able to exist by some alternate metabolic path in which this substance is not an essential factor. Growth in the absence of pyrimidine or thiamine is, however, extremely meager and of poor quality.

The partial replacement of thiamine by oxalacetate, shown in these experiments, suggests that this substance is able to function temporarily, at least, in the dismutation of pyruvic acid, a process

in which thiamine is normally involved. This is similar to the findings of Benham (**2**), who showed that oxalacetate was active for *Pityrosporum ovale,* an organism which is also stimulated by thiamine, and the findings of Smyth (**9**) who, in his study of thiamine-requiring strains of *Staphylococcus aureus* and *S. albus,* found that oxalacetate could replace thiamine for limited periods of time.

Stimulation by para-aminobenzoic acid of these *T. violaceum* strains cannot be explained by the present findings. The effect of the vitamin was slight and was not apparent unless doses as high as 78 to 300 micrograms were added to 5 ml. NH_4NO_3 broth. The PABA apparently acts independently of thiamine, since the addition of minimal effective concentrations of thiamine did not enhance its effect. Robbins (**7**) has described a strain of *Rhodotorula aurantiaca* which was completely deficient for both thiamine and PABA. Here, however, the sensitivity to PABA was much greater, a positive effect being observed, under the conditions studied, with as little as 0.000137 μg. PABA.

The requirement for l-histidine by one of the strains studied is the first instance of the requirement for a specific amino acid by one of the dermatophyte species. This strain was not, in contrast to the others, deficient for thiamine. It was also morphologically distinct. However, a single-spore culture from this strain produced a characteristic endothrix infection in a dog, and on several occasions developed sectors both morphologically and physiologically identical with the other *T. violaceum* strains studied. Such mutations, which probably occur frequently among the dermatophytes, may account for the large number of closely related species described in this group.

SUMMARY

1. *T. violaceum* grows slowly and poorly on Sabouraud's dextrose agar. The colonies are usually small, and glabrous, and consist of a mass of poorly developed mycelium and chlamydospores.

2. Of eleven strains studied on synthetic, chemically defined medium, ten showed a partial requirement for thiamine. The minimum effective quantity was 0.002 μg. in 5 ml. NH_4NO_3 broth.

3. Thiamine could be replaced for these strains by an equimolar amount of pyrimidine (2-ethoxy-methyl-6 amino pyrimidine).

4. Oxalacetate was found to substitute partially for thiamine under the conditions studied.

5. Growth of the thiamine-deficient strains was somewhat stimulated by comparatively large doses of para-aminobenzoic acid.

6. In the presence of maximum effective dosages of thiamine, further stimulation of growth was observed with the addition of casein or other peptones.

7. One strain (No. *365*) was morphologically and physiologically distinct from the others in that it grew rapidly on Sabouraud's dextrose agar, producing a white, fluffy colony with large numbers of microconidia. It required the amino acid, l-histidine, for growth and was not stimulated by either thiamine or para-aminobenzoic acid.

8. A single spore culture of strain No. *365* developed sectors which had morphological and physiological characters identical with those of ten other *T. violaceum* strains studied. Strain No. *365* is considered to be an unstable mutant form of *T. violaceum*.

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STUDIES IN THE GENUS CINTRACTIA. III. C. LEUCODERMA AND RELATED SPECIES

LEE LING

(WITH 2 FIGURES)

The name *Cintractia leucoderma* (Berk.) P. Henn., as applied in the current sense, represents a species-complex, reflecting considerable variation in both macroscopic and microscopic characters. Attempts made by several authors to split it have not been generally accepted. The present treatment, based chiefly upon the examination of types of all related species, classifies this group of fungi into three species and one variety.

One difficulty in studying this group of fungi is in the determination of hosts. The herbarium material usually consists of infected plants only, in which the development of inflorescences has been either inhibited or aborted. In several cases, specimens of such fungi were found to have been labelled as occurring on *Scleria, Cyperus, Carex, Scirpus,* or even *Glyceria.* Efforts to obtain experts' opinions on the correct identification of hosts have not always been successful, owing to the imperfect condition of the specimens involved. Extensive field studies will be necessary in order to decide whether these fungi occur on hosts other than *Rhynchospora,* especially on *Scleria.*

As in the previous papers of this series, the abbreviation following the citation of each collection indicates the institution where the specimen is located. Abbreviations which were not given in the previous papers are as follows: BM = British Museum (Natural History); G = Conservatoire et Jardin botaniques, Geneva. Wherever the location is not given, the specimen is in the writer's personal collection.

The writer wishes to express his thanks to the curators of various herbaria mentioned in this paper for their kindness in lending

him the specimens or permitting him to examine the material in their institutions; to Mr. J. A. Stevenson and Miss Edith K. Cash for help in preparing the manuscript; and to Dr. Charles Baehni for furnishing the photograph of the type of *Uredo scleriae* DC.

Cintractia leucoderma (Berk.) P. Henn. Hedwigia **34**: 335. 1895. (FIG. 1.)

Ustilago leucoderma Berk. Ann. Mag. Nat. Hist. II. **9**: 200. 1852.

? *Cintractia junci* (Schw.) Trel. f. *cylindrica* Wint. Hedwigia **26**: 11. 1887.

Cintractia affinis Pk. Bull. N. Y. State Mus. **67**: 28. 1903.

Cintractia amicta Cif. Ark. Bot. **23A** (14): 10. 1931.

? *Cintractia cancellata* Liro, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **6** (1): 7. 1935.

Cintractia leucoderma (Berk.) P. Henn. var. *chacoensis* Hirschh. Rev. Argent. Agron. **6**: 193. 1939.

Sori surrounding the culms and less often the branches of the inflorescence, usually inhibiting entirely the development of the inflorescence, extending to 4 cm. or less in length, 1–2.5 mm. in diameter, each covered by a thick, white false membrane which gradually flakes away disclosing a black, firmly agglutinate spore mass. Spores deep reddish brown to dark purple brown, usually subopaque to opaque, globose to oval, often cupped at one side, sometimes slightly angular, 13.5–19.5 μ, rarely up to 21 μ in length, verrucose, verrucae frequently obscure and sometimes confluent into short, irregular striae or cerebriform; epispore 1.5–2 μ thick; immature spores hyaline, gelatinous, invariably present in the inner layers of the sorus.

Material examined:

On *Rhynchospora cyperoides* (Sew.) Mart. Cuba: Santa Clara, June 5, 1940, W. L. White *229* (FH). Dominican Republic: Valle del Cibao, prov. Espaillat, Moca, in Cif. Mycofl. Doming. Exs. 5 (BPI).

On *Rhynchospora macrostachya* Torr. United States: Smithtown, Long Island, New York, Aug. 8, 1902, C. H. Peck and F. S. Earle, type of *C. affinis* (CH).

On *Rhynchospora pterocarpa* R. & S. (= *R. barbata* Kunth.). Brazil: Copacabana-Rio, in Rab.-Paz. Fungi Eur. & Extraeur. *4401* (BPI). Dominican Republic: Santo Domingo, Sabana de Guerra, in Cif. Mycofl. Doming. Exs. *6,* type of *C. amicta* (BPI).

On *Rhynchospora* sp. Argentina: Santa Fé, Lanteri, Feb. 1936, M. Job, type of *C. leucoderma* var. *chacoensis* (LP); Buenos Aires, La Plata, C. Spegazzini *3179,* sub *Cintractia junci* Trel. (LP). Dominican Republic: Santo Domingo, in Berk. Herb. *4735, type* (K). United States: Jacksonboro, South Carolina, May 29, 1907, C. E. Chambliss (BPI).

In the type collection of this species, the spores are dark purplish brown, subopaque, ornamented with rather obscure verruculations, and measure 13.5–19.5 μ in length. No other collections match it exactly.

The type of *Cintractia junci* f. *cylindrica* or *C. cancellata* is not available for study. But from Liro's redescription it leaves very little doubt that the name is a synonym of the present species.

Cintractia portus-argenti Cif., according to its original description, has a habitat identical with *C. leucoderma.* The type collection as represented both in the Stockholm Museum and in Ciferri's Mycofl. Doming. *84,* however, is identical with *Cintractia montagnei* (Tul.) Magn. var. *major* Desm., with sori located in the ovaries instead of the pedicels as described. Since it is difficult to tell whether the description was misprepared or the type specimen was mixed, the name can be treated only as a *nomen dubium* for the time being.

Cintractia leucoderma (Berk.) P. Henn. var. **striata** (Clint. & Zundel) comb. nov.

Cintractia striata Clint. & Zundel, N. Amer. Fl. **7**: 1029. 1939.

Habitat similar to the species. Spores ochraceous brown, nonopaque, globose to oval, 13.5–19.5 × 12–18 μ, prominently verrucose, verrucae arranged densely and regularly in semispiral striae, epispore 1.5–2 μ thick.

Material examined:

On *Rhynchospora cyperoides* (Sew.) Mart. (= *R. tracyi* Britt.). United States: Fort Pickens, Florida, May 31, 1903, S. M. Tracy

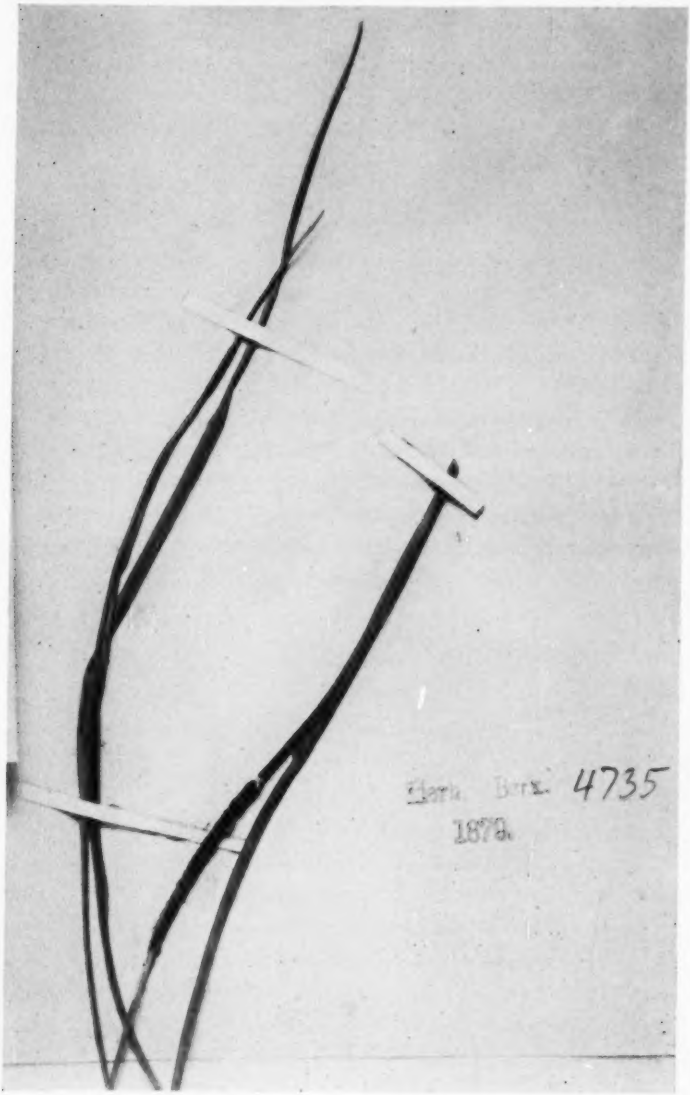

FIG. 1. *Cintractia leucoderma.*

8355, type (CH); Miami, Florida, May 7, 1904, P. H. Rolfs (BPI).

This form, as the description indicates, represents only an extreme in the arrangement of verruculations of spores which are also lighter in color than those of *Cintractia leucoderma*. It can be considered, at best, as a variety of the latter.

Cintractia pachyderma Syd. Ann. Myc. **22**: 282. 1924.

Sori surrounding the flower stalks, forming black, cylindrical, firmly agglutinate spore masses, extending to 1.5 cm. in length, 2–3 mm. in diameter, enclosed at early stages by a white, thick false membrane. Spores medium reddish brown, globose to broadly ellipsoid, 19.5–25.5 × 16.5–24 μ, ornamented with fine verrucae which are densely arranged into regular, parallel striae; epispore brittle; endospore with an evident central globule and a thick (3.5–6 μ), gelatinous wall.

Material examined:

On *Rhynchospora laxa* Vahl (= *R. corniculata* A. Gray). United States: Miami, Florida, in Seym. & Earle, Econ. Fungi *C104, type* (BPI).

As concerns the structure of spores, this fungus almost approaches the genus *Kuntzeomyces*. In this species, however, the epispore, though brittle, does not slough off to permit the endospore to pass out freely as happens in *Kuntzeomyces ustilaginoideus* P. Henn.

Cintractia scleriae (DC.) comb. nov. (FIG. 2).

Uredo scleriae DC. in Poiret, Encycl. Meth. Bot. **8**: 879. 1808.
Ustilago ? scleriae Tul. Ann. Sci. Nat. Bot. III. **7**: 89. 1847.
Cintractia krugiana Magn. in Engl. Bot. Jahrb. **17**: 490. 1893.
Cintractia krugiana Magn. var. *usambarensis* P. Henn. in Engl. Pflanzenw. Ost-Afr. Nachb. C. 48. 1895.
Cintractia leucoderma (Berk.) P. Henn. f. *utriculicola* P. Henn. Hedwigia **34**: 336. 1895.
Cintractia utriculicola Clint. Jour. Myc. **8**: 143. 1902.
Cintractia javanica Racib. Bull. Intern. Acad. Sci. Krakow **1909**: 351. 1909.

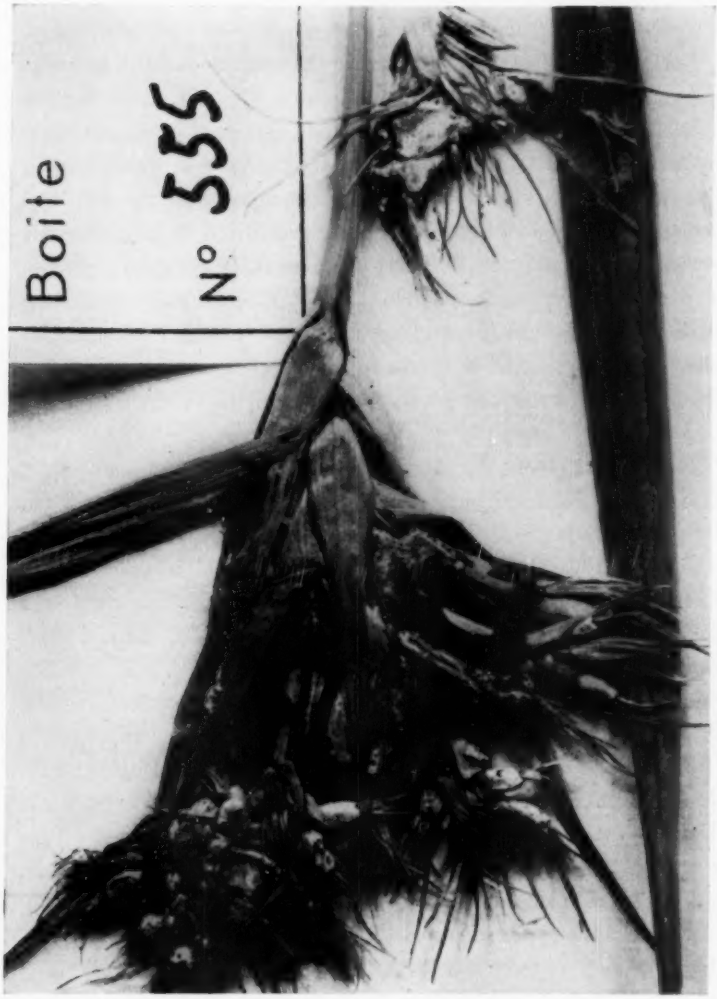

FIG. 2. *Cintractia scleriae.*

Cintractia usambarensis Cif. Ark. Bot. **23A** (14): 7. 1931.
Cintractia albida S. Ito, Trans. Sapporo Nat. Hist. Soc. **14**: 93. 1935.

Sori surrounding the pedicels and the branches of the inflorescence, oblong, occasionally oval or even subgloboid, extending up to 3.5 cm. in length, 2.5–4 mm. in diameter, each covered at first by a thick white false membrane enclosing a black, firmly agglutinated spore-mass; infection rarely spreading onto the scales of the rudimentary abortive inflorescences which are invariably present in the infected plants. Spores ochraceous brown, chiefly globose to oval, often cupped at one side, 12–16.5 μ, occasionally up to 18 μ in length, prominently verrucose, verrucae rarely confluent into short, irregular striae or cerebriform; epispore 1–1.5 μ thick; immature spores invariably present in the inner parts of the sorus.

Material examined:

On *Rhynchospora corymbosa* (L.) Britt. (= *R. aurea* Vahl). Australia: Brisbane, Queensland, Bailey, in Massee Herb. (NY). Belgian Congo: Kalikitim, R. P. Vantelborg (BR). China: Taipeh, Taiwan, May 1932, T. Suzuki, type of *C. albida*. French Congo: M. A. Chevalier, sub *Cintractia junci* Trel. var. *cylindrica* Wint. (FH). India: in Massee Herb. *5735* (NY). Indo-China: Saigon, Cochin-China, Aug. 24, 1889, K. Miyabe (CH); Cho-Ganh, Tonkin, Feb. 1923, A. Petelot. Indonesia: Soekanegara, Preanger, Java, 1900, M. Raciborski, type of *C. javanica* (FH); Tjikampek, Java, Apr. 1931, C. G. G. J. van Steenis (BPI). Malaya: Bakii-Junah Road, Singapore, 1892 (BM). Puerto Rico: Pueblo Viejo, Aug. 12, 1917, J. A. Stevenson (BPI). Tanganyika: Usambaren, Stuhlman *640*, type of *C. krugiana* var. *usambarensis* (BM, FH, NY).

On *Rhynchospora gigantea* Link. Paraguay: Antequera, Sept. 11, 1919, W. T. Bertoni *1151*, sub *Cintractia peribebuyensis* Speg. (LP). Puerto Rico: Sintenis *6672*, type of *C. krugiana* (NY).

On *Rhynchospora* sp. Colombia: Popogan, May 6, 1935, W. A. Archer (BPI). Cuba: in C. Wright, Fungi Cub. Wright *918* (FH). French Guiana: Cayenne, *type* (G). Mexico: Jalapa, in

Syd. Ustil. *224* (BPI). Venezuela: near Rio Tigre, Anzoategui, March 25, 1940, Agnes Chase *12541* (BPI).

This fungus has been commonly referred to as *Cintractia leucoderma*, probably due to general acceptance of Hennings' concept (**3**) that *C. krugiana* and its variety *usambarensis* are both synonymous with *C. leucoderma*. It is, however, easily distinguished from *C. leucoderma* by the location of the sori, which are always confined to the pedicels or the branches above the basal leaves, as well as by the lighter colored, non-opaque, smaller, and more evidently verrucose spores. In fact, the difference between these two is just as profound as between any two related species in this genus.

The name *Uredo scleriae* has been long neglected. Even Tulasne (**5**) had not examined the type when he transferred it into *Ustilago* with uncertainty. Fischer von Waldheim (**1, 2**), who had probably seen the type, published a brief re-description of it.

Should this species be considered a relative of *C. leucoderma* close enough to justify treating one name as a variety of the other, the epithet *scleriae* would have to designate the species. It not only has priority but it also represents a form more commonly occurring and more widely distributed than *C. leucoderma*. Such a treatment might increase the confusion.

As already discussed in a previous paper of this series (**4**), the name *C. leucoderma* f. *utriculicola* or *C. utriculicola*, which has been commonly but incorrectly used for an ovaricolous fungus, should be included in the synonymy of the present species.

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ROME, ITALY

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EXPLANATION OF FIGURES

FIG. 1. *Cintractia leucoderma* (type), showing spore mass surrounding the culm of *Rhynchospora* sp. × 1.

FIG. 2. *Cintractia scleriae* (type), showing sori confined to the pedicels of *Rhynchospora* sp. × 2.

GROWTH OF SAPROLEGNIACEAE IN SYNTHETIC MEDIA. II. NITROGEN REQUIREMENTS AND THE ROLE OF KREBS CYCLE ACIDS

HELEN SIMPSON REISCHER

The nitrogen requirements of saprolegniaceous fungi have been studied by Volkonsky (**1933**, **1934**), Leonian and Lilly (**1938**), Saksena and Bhargava (**1941**), Bhargava (**1945a**), and Whiffen (**1945**). A comparison of the results of these investigators leaves doubt as to whether ammonium salts can serve as a nitrogen source for any of these fungi, and, if so, to what extent ammonium nitrogen can replace other nitrogen sources. Volkonsky reported that ammonium acetate allowed very poor growth of *Saprolegnia* sp. and eight other species of Saprolegniaceae with cystine, but no growth of *Saprolegnia* sp. when H_2S was used as the source of sulfur. Leonian and Lilly stated that five members of this family grew well in a medium in which ammonium nitrate and L-cystine were the only added nitrogenous compounds. Saksena and Bhargava, and Bhargava found that either ammonium nitrate or sulfate as sole nitrogen sources supported good growth of five species in the presence of Na_2S (or K_2SO_4 for *Brevilegnia gracilis*); the side-effects of Na_2S on the medium appear to have limited growth where this compound was employed. Whiffen, however, reported that her isolates did not grow at all (with the exception of *Dictyuchus monosporus,* which made slight growth) with ammonium sulfate as nitrogen source even though the medium contained cystine as well. These investigators agree that nitrate nitrogen is generally unavailable to Saprolegniaceae, though *Brevilegnia gracilis* grew well (Bhargava, **1945a**) and *Dictyuchus monosporus* grew poorly (Whiffen, **1945**) on nitrate. There is also general agreement that amino acids, supplied singly as sole nitrogen sources, permitted much better growth than other nitrogenous compounds. Volkonsky found alanine, serine, phenylalanine, and cystine (or cysteine)

superior to other amino acids; Bhargava and Whiffen report that glutamic acid is the preferred nitrogen source.

The existence of organisms (tomato roots, Robbins and Schmidt, **1938**; certain *Neurospora* mutants, de la Haba, **1950**) which utilize nitrate nitrogen but not ammonium nitrogen for growth indicates that the metabolic path of inorganic nitrogen need not include reduction through ammonia. Nevertheless, ammonium nitrogen is generally used for synthetic purposes even by organisms such as the rat (see Schoenheimer, **1942**, and Lardy, **1949**) which has multiple specific amino acid requirements. The Saprolegniaceae do not require specific amino acids, but still do not seem to grow well or at all with ammonium salts as sole nitrogen source. Such fungi may transfer the bulk of organic nitrogen utilized without the formation of free ammonium ions by means of transaminase systems more extensive than the two (aspartic and glutamic transaminases) now firmly established (Cohen and McGilvery, **1949**), or they may resemble the *Neurospora* mutants of Fincham (**1950**) in being deficient in aminating ability only for amino acids participating in the established transaminase systems, so that any of a group of interconvertible amino acids will satisfy the growth requirement. Experiments designed to illuminate the nitrogen requirements and metabolism of a number of species of Saprolegniaceae were therefore undertaken.

The organisms investigated were *Achlya bisexualis* (female, JRR 355), *A. bisexualis* ? (male, JRR E 247), *A. colorata* (LS), *A. flagellata* (AZ), *A. Klebsiana* (LS), *A. racemosa* (G–S), *Brevilegnia unisperma* (LS), *Calyptralegnia achlyoides* (LS), *Dictyuchus monosporus* (DS–1), *Isoachlya intermedia* (IR–2), *Protoachlya paradoxa* (LS), *Saprolegnia delica* (LS), and *Thraustotheca primoachlya* (AZ). The basal medium used, and other details of experimental technique, may be found in a previous communication (Reischer, **1951**). C.P. chemicals were employed.

Achlya Klebsiana (LS), used in preliminary experiments, grew as rapidly and heavily in a glucose (1.0%) and NaH glutamate (0.2%) medium as in a medium containing gelatin hydrolysate (0.4%) and glucose, and as heavily and nearly as rapidly as in soluble starch-glucose-yeast extract broth. The use of glutamate was suggested by the reports of Whiffen (**1945**) and Bhargava

TABLE 1

THE EFFECT OF KREBS CYCLE ACIDS AND pH ON GROWTH OF *Achlya Klebsiana* (IN MINERAL BASE PLUS METHIONINE PLUS NH_4Cl, 0.01%). GROWTH ESTIMATED FIVE DAYS AFTER INOCULATION. SERIES A WITHOUT GLUCOSE, SERIES B WITH 0.25% GLUCOSE ADDED ASEPTICALLY AFTER AUTOCLAVING. THE FINAL pH IS GIVEN ON THE RIGHT HAND SIDE OF EACH COLUMN[1]

Addition	Initial pH											
	4.5				6.0				7.0			
	A		B		A		B		A		B	
None	0	4.5	+	4.1	0	6.0	++	4.1	0	7.0	+++	4.3
Na_2 succinate·$6H_2O$ 0.25%	+	8.1	+++	7.3	+	8.1	+++	7.8	+	8.2	+++	8.2
DL-malate 0.25% and $MgSO_4 \cdot 7H_2O$ 0.05%	±?	4.7	+++	6.0	±	6.7	+++	8.1	+	8.2	+++	8.2
Fumaric acid 0.25%	+	8.1	+++	7.5	±	8.1	+++	8.0	+	8.2	+++	8.0
trans-aconitic acid 0.25%	0	4.7	++	4.5	0	6.0	+++	6.2	0	7.1	+++	6.8
K_3 citrate·H_2O 0.25% and $MgSO_4 \cdot 7H_2O$ 0.1%	0	4.7	+++	4.5	0	6.2	+++	5.5	0	7.3	+++	6.5
Na H glutamate 0.25%	+	8.4	++++	7.5	++	8.4	++++	8.3	+	8.5	++++	8.3

[1] Determinations of pH were made with brom phenol blue, brom cresol green, brom cresol purple, brom thymol blue, meta cresol purple, and thymol blue.

(**1945a**) of the superiority of glutamate containing media. When 0.01% ammonium chloride or nitrate was substituted for NaH glutamate results varied with glucose concentration (see TABLE 4), with pH (see TABLE 1), and in different experiments from no growth at all to moderate growth.

Since glutamic acid is readily produced by a variety of organisms by the reductive amination of α-ketoglutaric acid, an attempt was made to substitute available acids of the Krebs tricarboxylic acid cycle for glutamic acid. *Achlya Klebsiana* grew well when ammonium salts (0.01%) and DL-malic acid (with the greater part of its activity as a chelating agent compensated for by the addition of 0.05% $MgSO_4 \cdot 7H_2O$ to that already present in the medium), Na_2 fumarate, or Na_2 succinate$\cdot 6H_2O$ were substituted for glutamic acid or NaH glutamate in the usual medium (mineral base, methionine, and glucose) (TABLE 1). The slight superiority of glutamate in table 1 (which was equally often not apparent in these experiments, see TABLE 2) is considered to be related to the limitations of the medium used (see Reischer, **1951**). Although succinate, malate, or fumarate alone permitted some growth, the effects of these acids and glucose on growth were clearly more than additive (see also TABLES 2 and 3).

The effects of *trans*-aconitic and citric acid (with 0.1% $MgSO_4 \cdot 7H_2O$ added to that usually present in the medium to substantially, though not completely, offset toxicity due to chelation of essential elements) in table 1 require further comment. The results of previous experiments employing Krebs acids had been either negative or inconclusive with respect to *trans-aconitate* and citrate in that their effects, if any, were slight and occurred under circumstances in which the buffering activity of these acids could have been solely responsible for the improvement in growth. Growth with glucose as sole substrate varied with the initial pH. Although one would not predict that *trans*-aconitate as such would show activity, one would expect equilibration with *cis*-aconitate, the form more stable in dilute aqueous solution at pH 7 to 10 (Ambler and Roberts, **1948**) and the form active in the Krebs cycle. In manometric experiments fumarate, malate, and succinate caused significant increases in oxygen uptake (over endogenous respiration) —these acids did not. Triethylcitrate and diethylsuccinate esters

were tested to obviate possible impermeability of the mycelium to citric acid. Both esters were toxic at concentrations down to 25 mg.% and lower concentrations had no apparent effect on growth, in the presence or absence of aseptically added ethanol or glucose.

TABLE 2

THE EFFECT OF SUCCINATE ON THE GROWTH OF *Achlya Klebsiana*. INITIAL pH 6.5. GROWTH ESTIMATED FOUR DAYS AFTER INOCULATION

Addition to basal medium plus methionine	Nitrogen source		
	None	0.01% NH_4Cl	Na H glutamate 0.2%
None	0	0	++
Glucose 0.5%	0	++	++++
Na_2 succinate·$6H_2O$ 0.25%	+	+	++
Glucose 0.5% and Na_2 succinate·$6H_2O$ 0.25%	+	++++	++++

TABLE 3

THE EFFECT OF SUCCINATE CONCENTRATION ON THE GROWTH OF *Achlya Klebsiana* (IN MINERAL BASE PLUS METHIONINE PLUS NH_4NO_3 0.01%). INITIAL pH 5.0. GROWTH WAS ESTIMATED AFTER FOUR AND SIX DAYS INCUBATION AT ABOUT 25° C

Additions	Substrate added					
	None		Ethanol[1]		Glucose[1]	
	4	6	4	6	4	6 days
None	±?	±?	±?	±	±	+
Na_2 succinate·$6H_2O$ 5.0 mg.%	±	±	±	+	+	+
Na_2 succinate·$6H_2O$ 10.0 mg.%	+?	±	+?	+	+	++
Na_2 succinate·$6H_2O$ 25.0 mg.%	+	+	++	++	++	+++
Na_2 succinate·$6H_2O$ 50 mg.%	+	+	++	+++	++	+++
Na_2 succinate·$6H_2O$ 100 mg.%	+	++	++	+++	++	+++
Na_2 succinate·$6H_2O$ 250 mg.%	+	++	++	+++	++	+++
Na_2 succinate·$6H_2O$ 500 mg.%	+	++	++	+++	++	+++

[1] 1.0% ethanol and 0.2% glucose added aseptically after autoclaving.

The place of aconitate and citrate in the internal economy of *Achlya Klebsiana* remains conjectural.

The chemical purity of commercially available synthetic succinate as compared to biologically produced acids, gives succinate great interest for the development of chemically defined media. The effect of succinate concentration on growth is shown in table 3. Acetic, benzoic, *p*-hydroxybenzoic, crotonic, gluconic, glutaric, gly-

colic, itaconic, sorbic, and tartaric acids, and ethanol were ineffective as substitutes for succinate.

These data are considered to provide evidence that the aerobic metabolism of the Saprolegniaceae proceeds through some variant of the usual pattern involving a Krebs cycle, though under conditions normally prevailing in the laboratory, at high concentrations of glucose, and at pH lower or higher than 7, growth is limited by some reaction related to the Krebs cycle. The partial nature of the block in metabolism suggested that a high carbon dioxide requirement might be the limiting factor. The activity of glutamate and succinate as substitutes for CO_2, noted by Lwoff and Monod (**1947**), could be predicted from the relation of all three compounds to the Krebs cycle (see Krebs, **1943**; Wood, **1946**). The results of a preliminary experiment to test this hypothesis were not entirely satisfactory, since *Achlya Klebsiana*, in a glucose-succinate medium, did not grow as well in a closed system (desiccator) with 5% CO_2 in air and 0.01 *M* bicarbonate as in the same medium without CO_2-bicarbonate. A definite increase in growth in a medium with glucose alone was noted in the desiccator with added CO_2 and bicarbonate, over growth in the same medium in a desiccator rendered more or less CO_2-free by the inclusion of a flask of KOH (25%) with a filter paper wick, but the amount of growth was disappointing here also.

Leonian and Lilly (**1940**) found that acetic, fumaric, glutaric, succinic, and tartaric acids stimulated, in varying degree, the growth of *Phycomyces blakesleeanus, Mucor ramannianus, Pythium ascophyllum,* and *Pythiomorpha gonapodioides.* The effect in the presence of poor nitrogen sources was explained as an "activation of ammonia." Such an activation would presumably occur by the amination of α-keto acids formed from the stimulating acids. The amino acids which were formed would then serve as good sources of nitrogen. The data in the tables of Leonian and Lilly indicate that aspartic acid or asparagine were poor sources of nitrogen inasmuch as growth in their presence was stimulated by succinic acid—an observation which is difficult to reconcile with a specific effect of organic acids on the utilization of inorganic nitrogen.

Achlya Klebsiana required glutamate for heavy growth; glycine or alanine were not effective substitutes as sources of organic nitro-

gen (see also Bhargava, **1945a**; Whiffen, **1945**). When organic acids are substituted for glutamate, *Achlya Klebsiana* required, for heavy growth, Krebs cycle organic acids specifically; acetic, glutaric, and tartaric acids were ineffective. Increasing concentrations of glucose, alone and with glycine, alanine, or acetate (TABLE 4)

TABLE 4

THE EFFECT OF INCREASING CONCENTRATIONS OF GLUCOSE, ALONE AND WITH ACETATE, ON THE GROWTH OF *Achlya Klebsiana* IN A MEDIUM CONSISTING OF MINERAL BASE, METHIONINE, AND NH_4Cl ONLY, AT AN INITIAL pH OF 6.5. GROWTH WAS ESTIMATED FIVE DAYS AFTER INOCULATION

Acetate concentration	Glucose concentration				
	None	0.1%	0.2%	0.5%	1.0%
None	0	++	++	+	±
Na acetate·$3H_2O$ 0.005%	±?	++	+	±	0?
Na acetate·$3H_2O$ 0.01%	±?	++	++	+	±
Na acetate·$3H_2O$ 0.04%	±	++	±	0	0
Na acetate·$3H_2O$ 0.1%	+	±	±	±?	0
Na acetate·$3H_2O$ 0.2%	++	±	0	0	0

progressively decreased growth as total substrate concentration increased. While a shortage of Krebs α-keto acids for amination would understandably limit growth, these data support the hypothesis that the primary deficiency is of CO_2 or a CO_2 substitute for maintenance of a Krebs cycle; utilization of inorganic nitrogen is a secondary effect. According to this hypothesis the decrease in growth at the higher concentrations of glucose is caused by side effects of an overflow metabolism such as the production of toxic acids or decrease in available energy because of incomplete oxidation. Swamping of oxidative mechanisms in high-carbohydrate (over 2.0%) media, resulting in the formation of a variety of organic acids later utilized, is characteristic of molds (Foster, **1949**). The Saprolegniaceae, aquatic phycomycetes, appear to be adapted to life in still more dilute media. That the addition of glutamate to a medium containing 1.0% glucose restores growth, suggests again that growth by these fungi is limited by a deficiency in a Krebs cycle component, possibly the CO_2 which is well established as a necessary part of this system. And, since glutamate restores growth, osmotic pressure can not be used as an explanation of the inhibition caused by 1.0% glucose.

When this investigation was extended to the other species of Saprolegniaceae listed earlier, similar results were obtained with all except *Brevilegnia unisperma*, *Calyptralegnia achlyoides*, and *Dictyuchus monosporus*. Variable, but never better than mediocre, growth occurred on glucose-ammonium salt media. The addition of succinate, malate, or fumarate to the medium yielded increased growth closely approximating or equal to that with added glutamate. The effects of organic acid and glucose were more than additive. Ammonium ion, but not nitrate ion, was an excellent source of nitrogen (TABLE 5).

TABLE 5

THE GROWTH OF NINE SPECIES OF SAPROLEGNIACEAE IN A MEDIUM CONTAINING MINERAL BASE, METHIONINE, Na_2 SUCCINATE·$6H_2O$ (0.25%), AND GLUCOSE (0.5%), AT AN INITIAL pH OF 6.5. SINCE PRELIMINARY EXPERIMENTS INDICATED THAT *Achlya colorata*, *A. racemosa*, *Isoachlya intermedia*, *Protoachlya paradoxa*, AND *Saprolegnia delica* DO NOT GROW WELL ABOVE 20° C, GROWTH WAS ESTIMATED AFTER SEVEN DAYS INCUBATION AT THIS TEMPERATURE

Fungus	Nitrogen source		
	None	NH_4Cl 0.01%	$NaNO_3$ 0.02%
Achlya bisexualis (female)	+	++++	+
A. bisexualis ? (male)	+	++++	+
A. colorata	+	++++	+
A. flagellata	+	++++	+
A. Klebsiana	+	++++	+
A. racemosa	+	++++	+
Isoachlya intermedia	++	+++++	++
Protoachlya paradoxa	++	+++++	++
Saprolegnia delica	+	++++	+
Thraustotheca primoachlya	+	++++	+

Calyptralegnia achlyoides became unavailable for further testing. *Brevilegnia unisperma* and *Dictyuchus monosporus* did not grow well until glucose was replaced by ethanol as a source of carbon and energy. These two species grew fairly well (in the usual medium containing mineral base, methionine, succinate, and ammonia) with acetate (0.25%, pH 7.0), glycerol (0.4%), lactate (0.25%), or gelatin hydrolysate (0.4%) as energy sources, but only slowly and irregularly on glucose (0.5%). All the other species investigated grew excellently upon glucose, starch, and glycogen, but not cello-

biose. Sucrose was equally well utilized by all except *Isoachlya intermedia, Protoachlya paradoxa,* and *Saprolegnia delica.* The following compounds did not cause significant increase over the slight growth supported by 0.25% succinate: l-arabinose, d-xylose, l-sorbose, pectin, glucosamine, gluconic acid, K 5-ketogluconate, and Na 2-ketogluconate. Since the addition of 0.5% glucose allowed the usual growth, none of these compounds was toxic. The restriction of carbohydrate utilization to glucose and glucose-containing compounds is in conformity with previous reports (Volkonsky, **1933**; Bhargava, **1945b**; Whiffen, **1945**).

SUMMARY

Eleven species of Saprolegniaceae make excellent growth with ammonium salts (but not nitrate) as nitrogen source provided that fumarate, malate, or succinate is also present. Other organic acids did not satisfy this requirement for *Achlya Klebsiana.* These organic acids, or glutamate, probably act as substitutes for CO_2 in the maintenance of the Krebs cycle of these organisms. Evidence of participation by citric and *cis*-aconitic acids in this cycle was not obtained.

While the majority of these species utilize glucose as a source of carbon and energy, *Brevilegnia unisperma* and *Dictyuchus monosporus* utilized glucose with difficulty but grew readily in the presence of ethanol.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Wm. J. Robbins, under whose direction this work was done, and Drs. F. Kavanagh and S. H. Hutner, for advice and encouragement. Thanks are also due Dr. J. R. Raper, Dr. L. Shanor, Dr. A. Ziegler, and Dr. E. K. Goldie-Smith, who generously contributed the isolates indicated by their initials.

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TWO HETEROTHALLIC SPECIES OF THE GENUS NIDULA

HAROLD J. BRODIE

(WITH 19 FIGURES)

Including the fungi discussed in this paper, at least one species has been studied in single-spore culture in each of the four known genera of the Nidulariaceae, *viz. Crucibulum, Cyathus, Nidula, Nidularia.* These studies have provided information on such subjects as: spore germination, inheritance of mating-type, mycelium color, fruit-body form, diploidization and nuclear migration in mycelia, etc. (**2**, **3**, **4**, **5**).

The genus *Nidula* appeared attractive as a subject for sexuality studies because it is so little known and because of its unique position in the family of the bird's nest fungi. *Nidula* was proposed by White (**8**) to include the two North American species *N. candida* (Peck) White and *N. microcarpa* Peck ex White, both of which are found along the west coast of North America from Oregon to British Columbia. *Nidula* is known also from the Himalayas and mountains in Japan. The writer has recently seen, in the Royal Herbarium at Kew, a collection of *N. microcarpa* found at 5000 ft. on Blue Mountain, St. Andrew, Jamaica, this evidently being the only known collection of the genus from the West Indies. These fungi would appear to be restricted to cool countries or to cool regions in warm countries.

The fruit-body of both North American species is stout, cup-like or crucible-like in shape and 0.5 to 1 cm. high. The cups are white, grey or pale brown and often have a strongly flared or recurved rim. The peridium consists of two layers, the inner thin layer being continuous over the mouths of young fruit-bodies. The peridioles lack funiculi, lie immersed in a gelatinous matrix and are adhesive. Thus *Nidula* resembles *Crucibulum* because of the structure of the cup and the epiphragm and resembles *Nidularia* because of the numerous unattached peridioles.

The two North American species may be readily distinguished by the characters set out below.

Nidula candida (FIG. 18)	*Nidula microcarpa* (FIG. 19)
Fruit-body 1 to 1.5 cm. high, grey to dark buff.	Fruit-body 0.5 to 1 cm. high, white to yellowish.
Rim strongly recurved at maturity.	Rim straight or but slightly recurved.
Peridioles moderately large, 1.5 to 2 mm. wide, light grey-brown, smooth.	Peridioles small, 0.5 to 1 mm. wide, red-brown, wrinkled.
Basidiospores 4–6 × 8–10 μ, elliptical.	Basidiospores 5–6 × 7–8 μ, elliptical to subglobose.

The culture for the first time of these two interesting fungi was made possible through the kindly cooperation of the Oregon Mycological Society whose members repeatedly sent both species of *Nidula* to the writer until germinable spores were found, and special thanks are due to Mrs. H. J. Oswald and Mr. Lindley Carson who sent specimens from Portland, Eugene and other localities in Oregon.

MATERIALS AND METHODS

Single-spore cultures were prepared following the technique used by the writer for other members of the Nidulariaceae (**2**). The occurrence of *Nidula* in cool regions suggested that spores might be expected to germinate readily at low or moderate temperature. A few spores were observed to germinate after 48 hrs. at 8° C. and at 15° C., but the percentage was too low to give sufficient cultures for study. When the writer's method (**2**) of heating spore suspensions at 40° C. for 48 hours was tried, a large proportion of spores germinated and individual spores were then cut out from dilution plates in the usual way.

During the course of the investigation, haploid and diploid mycelia were grown at room temperature (20–23° C.). For comparison, twenty haploids were kept at 8° C. for a period of six weeks. Growth was slower at the low temperature than at room temperature, but the mycelia which had been kept cold had formed thick cottony masses by the end of six weeks whereas the mycelia kept at room temperature had a sparse and appressed growth. A temperature of between 8 and 15° C. would therefore appear to be more favorable for the development of normal vigorous mycelium than higher temperatures.

Although no extensive effort has been made up to the present to induce diploid mycelia to fruit, no culture has shown any indication of fruiting on agar medium or on sterilized plant stems of various sorts.

NIDULA CANDIDA

Spores were obtained from a single peridiole from a fruit-body collected by Mr. L. D. Carson at 2500 ft. near Mount Hood, Loop Highway, Portland, Oregon, October 9, 1949.

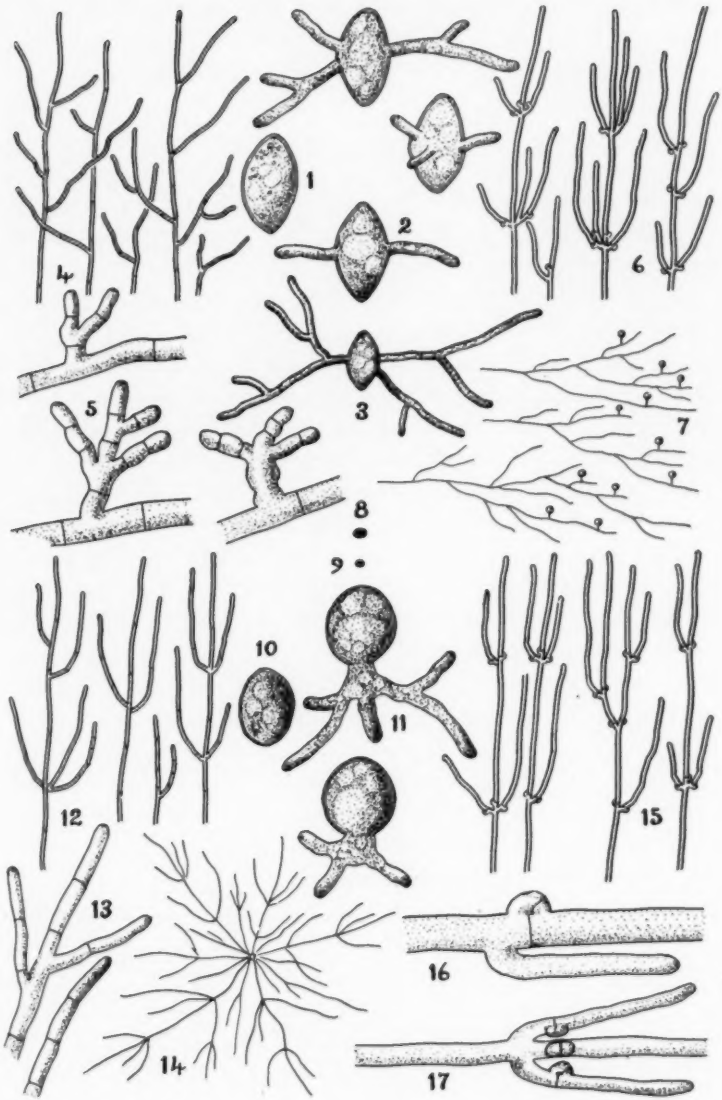
The basidiospores, which measured $5 \times 11\ \mu$ before germination, did not swell evenly as they germinated but had a tendency to change shape. The equatorial region became slightly distended which resulted in the spore assuming a spindle shape. From two to seven germ tubes arose around the equator of the spore (FIG. 2) and the latter became rapidly vacuolate as the hyphae grew outward.

Young mats of haploid mycelium measured about $150\ \mu$ in diameter at the end of three days and at this time the hyphae were still aseptate (FIG. 3). Septation occurred a day later. The haploids, which were snow white at first, consisted of delicate sinuous hyphae $3\ \mu$ in diameter. Transfers of two-month-old haploids were mostly white; a few showed traces of ivory or buff color. No pigmentation of the medium was produced by haploid mycelia of this species.

Oidia were developed in clusters on the ends of knobby upright branches (FIG. 5). In the moist atmosphere of Petri dishes the oidia were usually accompanied by a shiny exudate. The oidiophores surmounted by balls of exudate were reminiscent of the oidial structures of *Coprinus lagopus* Fr. described by the writer (**1**). Oidia are developed on mycelium of *Crucibulum vulgare* Tul. and *Cyathus striatus* Pers., but the writer (**2, 4**) has noted their absence in *C. stercoreus* (Schw.) DeToni and in *C. olla* Pers.

Twenty haploid mycelia one month old were paired in tubes in all possible combinations. Diploid mycelia appeared in certain pairings after ten days and, as a result of examination of all pairings, the mycelia were assigned to four sex groups as follows:

AB: 1, 2, 3, 6, 13, 19
ab: 5, 7, 9, 10, 12, 16, 20
Ab: 4, 15, 17
aB: 8, 11


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

Mycelium No. 14 proved by the analysis of its mating reactions to be a mixture of the genotypes (*AB*) and (*Ab*) and was doubtless the result of cutting out two spores inadvertently instead of one. A similar mischance probably produced Mycelium No. 18

FIG. 18. *Nidula candida.* Group of fruit-bodies showing flared rim and large pale peridioles characteristic of this species, × 2.5.

which was seen, soon after its isolation, to be diploid, the result of the union of two compatible haploids.

From the results of this analysis, it was concluded that *Nidula candida* is a heterothallic tetrapolar fungus.

The diploid hyphae produced in the pairings were 5 to 6 μ in diameter and provided with clamp connections. The branches fre-

FIGS. 1–8. *Nidula candida.* 1. Basidiospore before germination, × 1300. 2. Basidiospore germinating, germ tubes equatorial, × 1300. 3. Young haploid mycelium, hyphae still aseptate, × 700. 4. Leading hyphae of haploid mycelium, × 100 approx. 5. Oidia on knobby aerial branches, × 900. 6. Leading hyphae of diploid mycelium, × 100 approx. 7. Oidia in drops of exudate on haploid mycelium, × 50 approx. 8. Outline of single peridiole of *N. candida*, actual size.

FIGS. 9–17. *Nidula microcarpa.* 9. Outline of single peridiole, actual size. 10. Basidiospore before germination, × 1300. 11. Basidiospore germinating, germ tubes from end, × 1300. 12. Leading hyphae of haploid mycelium, × 100 approx. 13. Oidia on haploid mycelium, × 900. 14. Young haploid mycelium showing manner of branching, × 50 approx. 15. Leading hyphae of diploid mycelium, × 100 approx. 16. Clamp-connection and branch hypha on diploid mycelium, × 900. 17. Whorl of branches of diploid mycelium, × 650.

quently arose in whorls, giving the mycelium a characteristic appearance (FIG. 6). Some diploids were white, others very pale yellow, while still others were delicate grey lilac. No study has been made as yet of the inheritance of these colors.

NIDULA MICROCARPA

Spores were obtained from a single peridiole from a fruit-body collected by Mrs. H. J. Oswald, in mixed coniferous forest near Eugene, Oregon, Oct. 11, 1949. The spores measured $5 \times 7.5\ \mu$ before germination and swelled evenly during germination to about

FIG. 19. *Nidula microcarpa*. Group of fruit-bodies showing massive nature of cup and small, dark, wrinkled peridioles characteristic of this species, × 2.5.

$9\ \mu$. Germ tubes invariably arose at one end and developed from a kind of vesicle (FIG. 11).

Young haploid mycelia became septate 48 hours after germination of the spores. The hyphae were $4\ \mu$ in diameter and, in the manner of their branching, bore a closer superficial resemblance to diploid mycelium than do the hyphae of any other basidiomycete known to the writer. Several branches arose at a "node" and the hyphae were remarkably straight (FIG. 12).

Haploids were mostly white, a few were tinted with ivory or light buff. Even when quite young, however, most of them produced an intense pigmentation of the culture medium. The pig-

ment dissolved in the agar and the reverse of a culture was thereby colored a deep vinaceous brown (Ridgway, **7**). Of forty-six haploids, only four failed to cause pigmentation of the agar.

Most of the haploid mycelia showed a pronounced tendency to break up into oidia. The latter were borne on aerial branches but were not accompanied by any exudate such as that observed in *N. candida*. Also the oidia were longer and straighter than those of *N. candida* (FIG. 13).

Diploid mycelium was composed of hyphae ranging in diameter from 2.5–4 μ which tended to become associated into mycelial cords in old cultures.

Forty-six haploid mycelia were paired in all combinations in tubes and the pairs examined for diploid mycelium. The result was surprising: in only four tubes were diploids developed. The following pairs of mycelia were compatible: 7×40, 19×40, 30×32 and 34×32. The haploids involved in these pairings could be assigned to four mating types, as follows:

AB: 7, 19
ab: 40
Ab: 30, 34
aB: 32

The results offer no explanation for the behavior of forty mycelia of the series. Except the six listed above, all other mycelia failed to produce diploid mycelium regardless of how they were combined.

It is impossible to entertain the hypothesis that the fruit-body from which the spores were obtained might have been haploid, since six mycelia in the series did interact sexually.

The forty sterile mycelia may have been the result of a mutation since "sterile" mutants have been found among the Basidiomycetes. There is, at present, no way of investigating this possibility as it has not been found possible to induce cultures to fruit, thus precluding genetic analysis.

An effort is being made to secure a fresh sample of germinable spores of this fungus, but the search has not met with success as yet.

The regularity of the behavior of the mycelia which did interact sexually leads one to conclude that *N. microcarpa* like *N. candida* is heterothallic and tetrapolar but this conclusion must be drawn subject to confirmation by fresh analysis at a later date.

GENERAL REMARKS

A sufficient number and variety of fungi belonging to the Nidulariaceae have been observed in culture by the writer so that it is now possible to compare the different kinds. Diploid as well as haploid mycelia of *Crucibulum vulgare* Tul. and *Nidularia pisiformis* Tul. are fine-textured and individual hyphae are often markedly sinuous or even knobby. In addition, mycelia of these species grow slowly at room temperature. In contrast, the mycelia of five species of *Cyathus* thus far examined (in part, unpublished data) are coarse-textured and individual hyphae are mostly very straight. The mycelia of *Cyathus* spp. grow rapidly at room temperature.

The two species of *Nidula* appear to resemble *Crucibulum* and *Nidularia* in the general morphology of their mycelia which are fine-textured and slow-growing.

Both species of *Nidula* produce oidia on haploid mycelia. Among other members of the family, only *Crucibulum vulgare* produces oidia to a comparable degree. Oidia develop on the mycelium of *Cyathus striatus* but, as a rule, only in hanging-drop cultures and not on vigorous mycelium growing on agar plates.

Thus the study of *Nidula* in culture would appear to uphold the opinion of taxonomists (*e.g.* Lloyd, **6**) that *Nidula* is more closely related to *Crucibulum* and *Nidularia* than it is to *Cyathus*.

Also, the validity of the distinction between the two species of *Nidula* is upheld by the marked difference shown in culture by them. These differences are set out below.

N. candida	*N. microcarpa*
Basidiospore swells unevenly during germination and germ tubes emerge equatorially (FIG. 2).	Basidiospore swells evenly during germination and germ tubes arise from a vesicle at one end of spore (FIG. 11).
Haploid mycelium is regular in branching (FIG. 4).	Haploid mycelium is peculiar in branching, resembling diploid (FIG. 12).
Oidia are developed on haploid mycelium in balls of exudate (FIG. 7).	Oidia are developed on haploid mycelium but not in exudate (FIG. 13).
Haploid mycelia produce little or no pigment soluble in medium.	Haploid mycelia produce dark pigment soluble in medium.

SUMMARY

1. Two fungi of the genus *Nidula* have been studied in single-spore culture for the first time. These are the North American species *N. candida* and *N. microcarpa*.

2. Both species display the tetrapolar heterothallic pattern of sexuality as do all other members of the Nidulariaceae studied up to the present time.

3. The characteristics of the mycelia of *Nidula* appear to indicate a closer relationship to *Crucibulum* and *Nidularia* than to *Cyathus*.

4. *N. candida* and *N. microcarpa* display four major differences in the growth habits of their mycelia.

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STUDIES OF AN INSECT MYCOSIS. I. ETIOLOGY OF THE DISEASE [1]

ALFRED S. SUSSMAN

(WITH 3 FIGURES)

In the spring of 1947, a disease outbreak of some severity occurred among pupae of *Platysamia cecropia* L. which were being used for experimentation in the laboratory of Dr. C. M. Williams. The animals became infected after being operated on as described by Williams (**1946**, **1947**). Soon after infection, they blackened, lost their sensitivity to stimuli and finally died. In order to control the disease, it became necessary to investigate its nature and to isolate the causal agent.

During these investigations, it became apparent that this disease relationship afforded an advantageous combination of material for further research on the etiology and physiology of insect diseases. The parasite, *Aspergillus flavus* Link, is among the most thoroughly studied of the common molds, is easily cultivated and many strains are available. The host is also suitable for such experiments (Williams, **1947**) since the diapausing cecropia pupa is literally a "living test-tube" and is excellently suited for dissection and experimentation. Also, the brainless diapausing animal is in a relatively stable physiological steady state (Wigglesworth, **1948**), which allows for a maximum of repeatable physiological and biochemical testing. Therefore, it was felt that an investigation of the pathological relationship of this fungus and insect might throw some light on the fundamental biological problems involved in disease in general.

PRELIMINARY ISOLATION

The animals used in these experiments were either debrained or diapausing pupae which had been prepared by excising a few milli-

[1] Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Harvard University.

meters of tissue from the anal end, after which a plastic cover slip was fastened over the cut surface with a paraffin seal. Details of most of these procedures, and of the techniques used in "debraining" pupae, may be found in Williams (**1946**, **1947**). Figure 1 illustrates an animal prepared in this way. Blackening of the blood was prevented in two ways: in the first, a small crystal of

FIG. 1. Top: Brainless diapausing pupa of *Platysamia cecropia* with window prepared according to Williams (1946). Natural size.

Bottom: Comparison of the appearance of the interior of healthy and infected pupae of *P. cecropia* seen through posterior windows. *A*. Healthy animal showing the yellowish unspotted lobes of the fat body. *B*. Animal infected 24 hours previously by injection of spores. Note the spots which have appeared on the fat lobes. These animals have been treated with phenylthiourea in order to prevent darkening of the blood. Mag. 2×.

a copper reagent like phenylthiourea was placed in the body cavity of the insect before the cover slip was fastened in place; in the second, a few drops of a saturated solution of this compound in Insect Ringer's solution (Ephrussi and Beadle, **1936**) was introduced into the body cavity. These rather drastic operative techniques seemed to render the animals more susceptible to infection, for a large number blackened and died after the operation was concluded. It was from these animals that it was sought to isolate the pathogenic agent.

Such infected animals were first surface sterilized by washing with equal parts of a mixture of 95% alcohol and a 1 : 1000 mercuric chloride solution (Glaser, **1927**). The residual mercury salt was removed by rinsing the animals in sterile distilled water, after which they were slit open inside Petri dishes containing appropriate nutrient media; the blood and dissected pieces of tissue were then streaked over the surface of the medium used for the growth of the pathogen.

The media used included potato slices, in case the pathogen was *Entomophthora*-like in its nutrition (Sawyer, **1929, 1931**), or yeast and malt agar, in case the organism was a common fungous or bacterial pathogen. These plates were incubated at room temperature and pure cultures of each of the organisms found were established on yeast and malt agar slants.

In order to determine which of the organisms isolated were pathogenic to the animal, suspensions of each of the suspected organisms in sterile distilled water were introduced directly into the body cavity of healthy pupae, after which the plastic cover slip was sealed in place. The animals were held at room temperature and the effect of the various inocula was observed periodically.

The diseased pupae from which the original isolations were made all yielded the same microorganisms. These appeared within three days after inoculation and incubation at room temperature on all the media used. Two species of *Aspergillus, A. flavus* Link and *A. luchuensis* Inui [2] were obtained in pure culture, as well as a Gram negative rod and an unidentified yeast. The pathogenicity of each of these isolates was tested as before and it became apparent in

[2] The author is indebted to Dr. Kenneth Raper of the Northern Regional Research Laboratory for making these identifications.

less than a week after infection that only the two species of *Aspergillus* could develop within the pupa and ultimately cause its blackening and death. Figures 1 and 2 show the appearance of such an infected animal. Upon infection, it gradually lost the ability to move its abdomen, failed to respond to stimuli, and then blackened

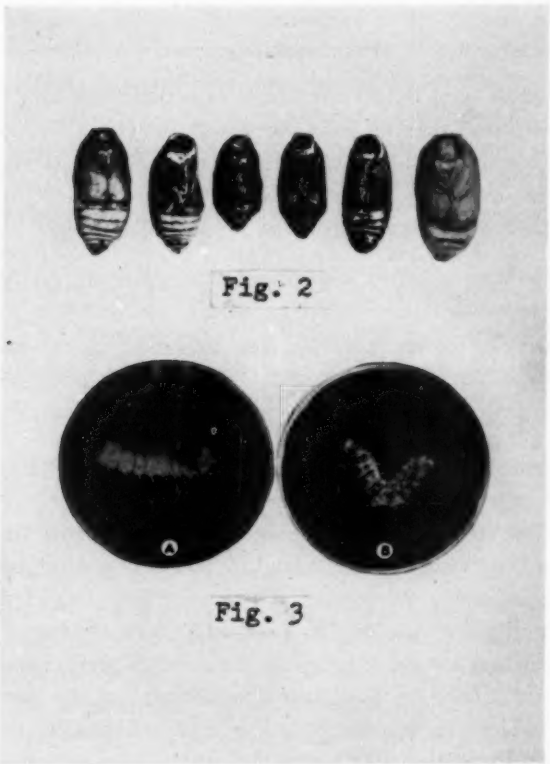

FIG. 2. Comparison between the appearance of brainless pupae of *P. cecropia* infected with *A. flavus*, and uninfected control. The animals at either end are control uninfected animals. The others are infected and show the characteristic shrunken appearance and blackening of the integument and blood. App. ½ nat. size.

FIG. 3. Fourth instar larvae of *P. cecropia*. *A*. Healthy uninfected animal. *B*. Animal infected 48 hours previously by the dusting of spores of *A. flavus* over its integument. Note the limpness and spotted appearance characteristic of the diseased animals. App. nat. size.

and died. It was noted that *A. flavus* was somewhat more virulent than *A. luchuensis* since the latter organism occasionally failed to develop because of being walled off from the interior of the host by hemolymph cells and epithelium. This rarely occurred with *A. flavus*. Both organisms were reisolated from the diseased animals, so that Koch's postulates were entirely fulfilled.

INFECTION OF LARVAE

The larvae used were reared from eggs that were kept in wide-mouthed bottles covered with cheesecloth or with a loosely fitting cap. Food was supplied chiefly by leaves of *Prunus serotina*, and, to a lesser extent, by leaves of *Salix* sp.

Inoculation was accomplished in the following ways:

1. Injection of spore suspensions into the body cavity of the animal.
2. Spraying of spore suspensions over the integument of the animal.
3. Dusting of spores over the integument of the animal.

After inoculation, the insects were incubated at 25° C. and observed daily for signs of infection.

The first technique used was the injection of a spore suspension into the body cavity of the animal by means of a 1 ml. tuberculin syringe and a No. 27 needle. To prevent wriggling of the larva upon insertion of the needle, with subsequent ripping of the integument and excessive bleeding, the animals were anaesthetized with CO_2. Loss of blood, upon withdrawal of the needle, was prevented by plugging the wound with melted paraffin.

It was found that 100% infection was always obtained by this means; on the other hand, controls, injected only with sterile distilled water, survived. Diseased larvae became more spasmodic in their movements, began to void liquid feces, and developed bluish-black spots under the integument. They soon lost their sensitivity to stimuli like pin pricks, became limp and flaccid, stopped feeding, and soon died. Figure 3 illustrates the appearance of diseased larvae compared with healthy ones.

Next, spray infections of larvae were carried out using a De Vilbiss atomizer and spore suspensions in water and in a sodium oleate-gelatin mixture. The experimental animals were thoroughly sprayed with the spore suspension, after which they were allowed to dry in air before their return to the rearing chambers. Anderson and Henry (**1946**) used this combination of a wetting and adhesive agent in order to increase the effectiveness of the fungous inoculum they were using for infecting plants, while Notini, Mathlein and Lihnell (**1944**) used green soap as a spreader for green muscardine of *Galleria* and other insects. In testing the efficacy of water and of the sodium oleate-gelatin mixture, controls were run with each series, using a sterile water spray in the first case, and a sterile sodium oleate-gelatin spray in the second. The results showed

TABLE 1

RESULTS OF AN EXPERIMENT USING A SODIUM OLEATE-GELATIN MIXTURE AS A SUSPENDING MEDIUM FOR SPORES OF *A. flavus* IN ORDER TO INFECT LARVAE OF THE CECROPIA MOTH BY SPRAYING

Treatment	No. of animals used	No. dead after 1 week	% killed	Statistical significance	
				Chi-squared	Probability
Inoculated	24	15	63	9.50	Less than 0.01%
Control	12	1	8		

about 50% infection for the animals sprayed with the spore suspension in water and 63% for those sprayed with spores in the sodium oleate-gelatin mixture. Table 1 furnishes the data for a typical experiment using the latter mixture as a suspending medium. The sodium oleate-gelatin mixture was quite effective as a base for the distribution of fungous spores, while water alone proved somewhat less so.

Finally, dusting experiments were carried out as suggested by Lefebvre (**1934**) and Boyce and Fawcett (**1947**), who rolled the animals over the surface of Petri dish cultures of the pathogens to insure the spread of an abundance of spores over the insect's integument. In order to demonstrate that smothering was not responsible for the death of the animal, controls were rolled on sterile agar, and also over spores of a non-pathogenic fungus,

Penicillium notatum (Westling). In some cases, a camel's hair brush was used to dust spores over the animals. As a result, it was found that it was possible to infect the larvae in this way, since animals inoculated with spores of the pathogen developed typical symptoms of the disease, while the controls remained healthy.

It is apparent that the larval stage of *Platysamia cecropia* is exceedingly susceptible to infection by *Aspergillus flavus*. Each of the methods of inoculation used, injection, spraying and dusting, produced the disease. Injection of spores directly into the body cavity of the host proved to be the most effective, since 100% infection could be obtained in this way. Burnside (**1930**) found this to be the case in his experiments with certain *Aspergillus* diseases of bees, although a few larvae were infected by direct penetration of the integument. This agrees with the writer's results, suggesting that *Aspergillus* infections of bee larvae may follow a course similar to that in the cecropia moth. Analysis of the method of entrance used by the fungus, and its subsequent history in the animal, will be made in a subsequent paper, wherein the histopathology of the disease will be reported.[3]

INFECTION OF PUPAE

The rather drastic treatment used for the initial infection of pupae of *Platysamia cecropia* gave no information as to the mode of entrance of the fungus or as to whether or not the fungus could attack the pupae under natural conditions. Therefore, a series of investigations were undertaken using inoculation techniques which more nearly simulated natural conditions.

The inoculation methods outlined in the section on "Preliminary Isolation" were modified in a series of experiments whereby healthy, previously chilled, diapausing pupae were inoculated by injection, spraying and dusting. These techniques were the same as those used for the infection of the larvae of *P. cecropia*, with only a few modifications. Thus, in the injection experiments, the syringe needle was inserted laterally into the intersegmental membrane of the second thoracic segment, whereupon the spore sus-

[3] Accepted for publication in the Annals of the Entomological Society of America.

pension was discharged into the animal's body cavity. In this way, excessive loss of blood was prevented and anaesthesia of the pupae was found unnecessary since no movement of the pupal thorax was possible. Otherwise, the techniques used for inoculating the pupae were identical with those used for the larvae.

The minimum lethal dose (MLD) (as defined by the American Phytopathological Society, **1943**) was estimated by using infection as a measure of the virulence of the pathogen. To accomplish this, spores of a two-week-old culture of *A. flavus,* grown on malt agar, were used. These spores were suspended in distilled water and their number per ml. was counted in a Levy-Hauser counting chamber. Spore suspensions were divided into two lots and each was serially diluted in sterile distilled water to yield spore loads of 10,000, 1,000, and 100 spores per ml. 0.1 ml. of each spore suspension was injected into pupae of *P. cecropia,* after which the animals were incubated at 25° C. and observed daily for signs of infection.

TABLE 2

DETERMINATION OF THE MLD OF SPORES OF *Aspergillus flavus* LINK NECESSARY TO PRODUCE INFECTION IN PUPAE OF *Platysamia cecropia* L.

Dosage	Spore load per dose	Results one week after inoculation	
		No. dead	No. surviving
0.1 ml.	1000	2	0
0.1 ml.	100	1	1
0.1 ml.	10	0	2

In the injection experiments, using massive inocula, 100% infection of pupae was obtained at room temperature, while control animals, injected with the same amount of sterile distilled water, survived and remained healthy. Using this method of inoculation, the MLD was investigated for spores of *A. flavus.* The results (*cf.* TABLE 2) indicate that the MLD for two-week-old spores of this strain of *A. flavus,* growing on malt agar, is approximately 100 spores. It was not thought profitable to define this figure more precisely since there is so much variation in both the experimental animals and in the pathogen. However, it may be possible in

subsequent experiments to use the MLD as a quantitative measure of virulence of related species of fungi toward various insects.

The pupae could not be infected by spraying since the animals survived inoculation with a heavy spore suspension. Similarly, dusting spores over the integument with a camel's hair brush or rolling the animals over a Petri dish culture of the fungus gave negative results.

Evidence would suggest that the resistance of the pupa to infection by spraying or dusting of spores is due, in part, at least, to the waxy epicuticular layer of the insect's integument which acts as a protective film. For example, Ludwig (**1948**) suggested that the epicuticular lipid film protected insects from attacks by parasitic fungi but did not perform any experiments to verify this. Kühnelt (**1928**) demonstrated that the removal of the lipids of insect cuticles decreased the ability of these animals to withstand desiccation. The epicuticle can be removed by the following:

1. Organic solvents like chloroform and ether (Wigglesworth, **1945**; Ludwig, **1946, 1948**; Klinger, **1936**; Umbach, **1934**).
2. Abrasive inert dusts like alumina (Beament, **1945**).
3. Emulsifiers and detergents like lecithin (Hurst, **1940, 1941**; Wigglesworth, **1945**).

Consequently, in order to determine if it was the lipid epicuticular layer that was instrumental in preventing infection by *A. flavus*, the waxy coating of the insect was removed by suspending the animal for 5 minutes in ether. Control animals treated in this way survived and matured normally. Then, these "de-waxed" animals, which were thoroughly rinsed with water and dried, were rolled over a Petri dish culture of *A. flavus* and incubated at room temperature. Other animals, not treated with ether, were inoculated in the same way. Also, in order to prove that smothering by spores was not a factor causing death of the insects, "de-waxed" animals were dusted with spores of *Penicillium notatum*, a non-pathogenic form, while still other "de-waxed" controls were rolled over sterile agar. None of the controls was killed by the fungus and, in fact, the animals were able to mature and produce viable adults. Results of a typical experiment are set forth in table 3. It is at once seen that there is a significant difference in the amount of infection

of "de-waxed" and control animals. This would suggest that the lipid layer of the epicuticle of the pupae serves as a protective barrier which prevents either the germination of spores or the penetration of the pathogen's hyphae.

In direct contrast to the course of infection in larvae of *Platysamia cecropia*, it was impossible to infect the pupae by spraying or dusting spores over their integuments. To accomplish infection it was necessary to employ rather violent techniques, such as injection of spores by a syringe or direct transfer of spores into the body cavity through the posterior window. Part of the reason, at

TABLE 3

THE EFFECT OF "DE-WAXING" OF PUPAE OF *Platysamia cecropia* L. UPON THE INFECTIVITY OF *Aspergillus flavus* LINK

Treatment	No. animals inoculated	No. animals surviving	Statistical significance	
			Chi-squared	Probability
"De-waxed" and inoc. with *A. flavus*	10	5	6.67	Less than 1%
Control inoc. with *A. flavus*	10	10		

least, for the resistance of pupae to infection through their body surface is suggested by the results given in TABLE 3, which indicate that dissolution of the epicuticular layer makes the animal more susceptible to attack by spores of *Aspergillus flavus*. These results are in agreement with those of Metalnikov (**1941**), who used an adjuvant and an adhesive in the dissemination of bacteria which attacked the grape weevil, and with those of Notini *et al.* (**1944**), who used green soap in order to spread the muscardine disease of *Ephestia*.

The results of previous experiments with larvae support the suggestion that it would be valuable to use the adhesive and sticker, as do the experiments with "de-waxed" pupae. Therefore, it is suggested that the use of detergents or inert dusts in the dissemination of fungous spores for the biological control of insects is indicated, at least in those regions where the "saturation" point for the pathogen has not yet been reached (Sweetman, **1936**; Fawcett, **1944**).

INFECTION OF THE ADULT

Because of the ephemeral nature of most adult Lepidopterans, there is very little known about their disease relationships. Thus, in order to investigate some of the aspects of this problem, and to complete the survey of the effect of *A. flavus* upon the various stages in the life history of *P. cecropia,* infection of the adult moth was attempted.

The animals were reared from pupae and were kept in wire cages at room temperature. Inoculation was accomplished by spraying an aqueous spore suspension over the body surface of the animals, whereas control animals were sprayed only with sterile distilled water.

It was found that 100% infection resulted within 4 days after spraying, while control animals survived. The disease manifested itself by the gradual weakening of the animals, so that they could not raise themselves off the bottom of the cage. Upon the death of the animals, the fungous mycelium spread over the thorax and abdomen and, with the onset of sporulation, soon produced a green felt over the moth's body.

GENERAL CONCLUSIONS

With the demonstration that adults of *Platysamia cecropia* are susceptible to infection by *Aspergillus flavus,* it is seen that all stages in the life cycle of the moth, after hatching, can be attacked by this fungus. Several inoculation techniques were used and it was apparent from the results that there are differences in the manner in which the disease may be transmitted to the various stages in the life history of the insect.

In contrast to the relative ease with which the larvae can be infected, pupae can get the disease only by direct injection into the body cavity. The resistance of the pupa to infection via the integument has been traced to the presence of a lipid protective layer on the epicuticle which presents an effective barrier to infection from outside. Such resistance is probably of great importance to the animal in nature, offering as it does a valuable supplement to the humoral and cellular immune factors used in its defense against infection. However, once the parasite is introduced into the body cavity of the pupa, the susceptibility of the host becomes manifest

since the fungus attacks and kills the pupa in the same way that it infects larvae.

It is apparent, then, that although there may be certain mechanical barriers to the entrance of *A. flavus* into pupae, once these barriers are breached and the fungus enters the body cavity of the host, infection will result. This would indicate that the pathogen belongs to that category of parasite which, although it can live saprophytically, will be "parasitic, or semi-parasitic, whenever spore or mycelium comes in contact with the insect—and under suitable conditions may kill it" (Fawcett, **1944**).

The author would like to express his gratitude to Dr. William H. Weston, under whose direction this work was done, and to Dr. Carroll M. Williams, whose help was invaluable in providing techniques and material for these investigations.

SUMMARY

1. The causative agents of a disease affecting the giant silkworm, *Platysamia cecropia* L., have been isolated and identified as being *Aspergillus flavus* Link and *A. luchuensis* Inui.

2. Infection of each of the stages of the life history of the insect has been possible using *A. flavus*.

3. Although the insect possesses mechanical barriers to infection, once the fungus penetrates these, infection results.

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A MORPHOLOGICAL BASIS FOR CLASSIFYING THE SPECIES OF AURICULARIA

BERNARD LOWY

(WITH 1 FIGURE)

The genus *Auricularia* is well known and readily recognized, but the species are very imperfectly understood. Saccardo (**10**) lists over fifty species, while Holtermann (**5**) and Möller (**8**), at the other extreme, suggest that all tropical Auriculariaes belong to a single widely distributed and extremely variable species. Most students of the group now believe that there are several species in the genus, but attempts to distinguish them have not been notably successful.

Barrett (**2**) attempted a treatment of the North American species. Kobayasi (**6**) has recently monographed the species of eastern Asia. Both of these students have relied almost completely upon certain superficial features such as color, size and configuration of the hymenial surface. These characters, however, vary according to the age of the specimen, exposure to light, availability of moisture and other environmental factors. As a consequence of the utilization of such criteria, great confusion has resulted. It should be noted, however, that these characters, though superficial, are not entirely without value, but should be placed in a position of secondary importance, retaining them where experience has shown their use to be justifiable.

In the present treatment, I have regarded color, shape and size as subordinate characters and have chosen as the chief criterion the internal structure of the fruiting body, which shows a uniformity not affected to a significant degree by the vicissitudes of the environment.

The concept of the genus here adopted is that accepted by Martin (**7**) which includes in *Auricularia* the species sometimes assigned to *Hirneola*. It is believed that even for those who prefer

to regard *Hirneola* as distinct, the features here emphasized will retain their value.

It has been recognized, since Burt's (3) original description of *A. rosea*[1] in 1921, that the presence of a very prominent medullary layer is always associated with that species and serves as one of its most easily identifiable traits. As the examination of other species progressed, it became evident that some structural modification of the context could always be detected. After study of a large number of specimens from widely scattered sources, it was found that all of them could be placed into one of two groups: those which always showed a distinct medullary layer in section and those which had no layer of this kind or, at best, one which was only poorly defined. Moreover, the forms having a medullary layer showed a distinct stratification of hyphae, of which the medulla constituted a well-defined zone, usually central in position. Because of the different patterns of zonation to be found among the species and the fact that they are constant and easily recognizable, their special value in a taxonomic scheme is apparent. These facts, which have not heretofore been utilized to their fullest advantage, are exploited in this paper and will be discussed in detail.

The color of the fruiting body has been considered by all previous workers in making specific determinations. This practice has been employed, however, not in recognition of the special validity of this feature, but rather as a result of the paucity of other pertinent data. It is known that the color of a specimen as found in he field is affected by at least two conditions in its environment; its access to light, which is manifestly variable, being dependent upon the fortuitous location of the fungus, and the age of the specimen. Although color is a striking feature of some Auriculariales, the variability of this factor for a given species is such as to suggest that it can be of limited value in taxonomic work. It has often been observed that the fruiting bodies of a single species growing on the surface of a log may show varying degrees of pigmentation according to the intensity of light to which they

[1] *A. rosea* is antedated by *A. fuscosuccinea* (Mont.) Farlow, Bibl. Index 307. 1905. The specific epithet was applied by Montagne to the same fungus from the West Indies in 1841.

happen to be exposed. Burt (3), describing *A. rosea,* states that it has "a shell-pink color and texture of a rose petal when growing," but adds that in drying, it becomes "vinaceous and at length deep brownish drab." It was approximately in the latter condition when I examined the type specimen (29 years after its collection), but it is doubtful whether any two observers would agree as to the color designation which would best describe it in its present state. It is not unreasonable to suppose, therefore, that certain errors have been introduced in the interpretation of some species where too much reliance has been placed upon color differences. This is true even though the Ridgeway standards have been extensively used. It should be added, in the instance just cited, that upon sectioning the fruiting body, its morphology was found to be identical with that of other specimens of *A. rosea* which had been recently collected.

In order to describe adequately the relationship of various hyphal elements in the mature fructification, it was necessary to introduce a special terminology based upon the characteristic zonation of the hyphae. This can best be explained by the means of illustrations showing transverse sections through basidiocarps comparing the two general types under consideration.

A description of each of these zones follows.

Zona pilosa. The sterile, superior, pilose zone is present in all species and, though showing considerable variation for the genus as a whole, is relatively constant for each species. The hairs may be short or long, wide or narrow, forming a dense matted layer or be more widely scattered; the tips may be either blunt or pointed and the entire hair either hyaline or colored. The character of the hairs, taken by themselves, is certainly not a sufficient criterion to allow a diagnosis of a species to be made, but it is often very suggestive and, within rather wide limits, quite useful. For example, a specimen having hairs over 500 μ long and densely matted, as in *A. polytricha,* presents a striking contrast with a specimen having hairs about 100 μ long and not densely matted, as in *A. rosea.*

Zona compacta. This zone is found in all species and may be identified as the one from which the hairs arise. It is composed of a very dense, narrow zone of hyphae about 3–5 μ in diameter,

generally oriented perpendicular to the abhymenial surface, the individual elements being separated from each other only with great difficulty.

Zona subcompacta superioris. The hyphae of this zone are more loosely arranged than in the compacta, are about 3–7 μ in diameter, and generally perpendicular to the surface.

Zona laxa superioris. This is a very conspicuous zone in those species having a medullary layer, in which forms it is located immediately above the medulla. The term "laxa" is intended to be descriptive of the most characteristic property of the hyphae composing it. That is, their relatively loose, reticular arrangement, forming numerous anastomoses, allows for the identification of individual elements. The hyphae generally vary from about 3–8 μ in diameter.

Medulla. This zone, the presence of which in some species gives the fruiting body its most characteristic appearance in cross section, is composed of hyphae having a diameter of about 6–10 μ, is always centrally located with respect to the hymenial and abhymenial surfaces and traverses a course parallel with these throughout the fruiting body. In *A. tenuis,* the medulla is composed of two distinct parallel strands horizontally oriented and separated from each other by a band of loosely arranged hyphae.

Zona laxa intermedia. In species without a medulla, there is no distinction between a superior and an inferior lax zone, the hyphae occupying the central region of the fruiting body, between the compact zones, being organized into a single lax zone oriented predominantly parallel with the surface and having a diameter of about 5–10 μ.

Zona laxa inferioris. In species having a medulla, this zone has the same characteristics as the zona laxa superioris. It is lacking in those species without a medulla.

Zona subcompacta inferioris. In species having a medulla, as well as in those without one, this zone has the same characteristics as the zona subcompacta superioris.

Hymenium. This is a dense, gelatinous layer inferior in position, bearing 4-celled hypobasidia from which the epibasidia originate, giving rise in turn to the sterigmata which eventually pene-

trate the tough membranous surface and bear cylindrical or allantoid basidiospores at their tips.

Since the term "context" is not always interpreted in the same way by all authors, I have employed it here in the sense of Ainsworth and Bisby (1), to include all zones excepting the hymenial and the pilose.

It may be argued that a maze of terminology already exists and that no constructive purpose is served by increasing this still further. I believe, however, that the analysis of *Auricularia* presented in this paper justifies this course and that a clarification is attained by the new usage which would not be effected without it.

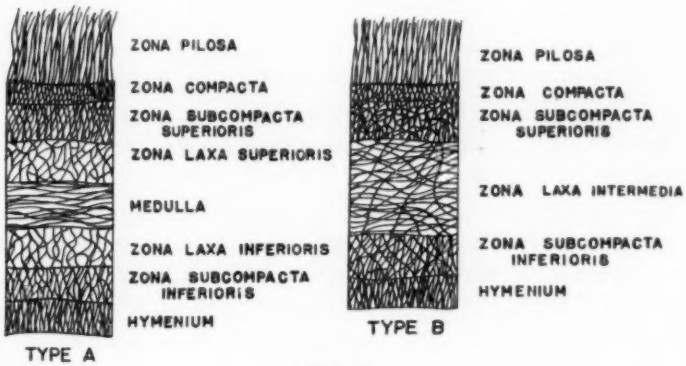

FIG. 1.

This is not to say that the invention of terms has automatically elucidated the issues, but rather that a situation was found which required exact description and that no existing terminology suited its needs. In a paper by Overholts (9) on the taxonomy of the Hymenomycetes and in another by Corner (4) on *Polystictus xanthopus,* some suggestions were made which were taken into consideration before deciding upon the new terminology. Corner has set up a taxonomic scheme based upon hyphal differences and it was believed that it might have provided an adequate framework for *Auricularia* as well, but this did not prove to be the case. Overholts notes the existence of discrete zones of hyphae in a number of Hymenomycetes and their absence in others. Regarding the utilization of internal structure as a means of identifying

a fungus, he says: ". . . identify on the basis of external appearance where that can be done with certainty, but in cases of doubt, however small, do not hesitate to allow the facts of internal structure to decide the case." *Auricularia* is such a case and it has proved fruitful to make use of internal differences in separating the species. On the other hand, the classification of hyphae suggested by Corner, though histologically of great interest and certainly applicable to some fungi, did not lend itself with facility to the solution of this particular problem. The critical point of this discussion is that in making an identification using sectioned material, we should try to deal with hyphal configurations as they appear to us in the context, rather than with the individual elements composing it.

A key to the species of *Auricularia,* based upon the morphological characters discussed, is given at this time. The names used are those which I believe have priority according to the Rules, although a discussion of the nomenclatural issues is being deferred until a later date.

The key is the result of the study of a large number of specimens of each species which were made available, for the most part, from the collections of the State University of Iowa, the New York Botanical Garden, the Missouri Botanical Garden and the Bishop Museum in Honolulu. My thanks are due Dr. D. P. Rogers of the New York Botanical Garden, Dr. C. W. Dodge of the Missouri Botanical Garden and Miss M. C. Neal of the Bishop Museum, who gave me access to the *Auricularia* collections of their respective institutions. Specimens from Panama were also studied, including many which I collected on Barro Colorado Island, and it is with pleasure that I acknowledge the courtesies extended to me by Mr. James Zetek, Director of that research station. This work was suggested by Professor G. W. Martin and done under his supervision.

KEY TO THE SPECIES OF *Auricularia*

a. Context with a distinctly differentiated medullary layer (FIG. 1, A).....b
a. Context without a distinctly differentiated medullary layer (FIG. 1, B)...f
b. Abhymenial hairs 3–5 mm. long; medullary layer 90–100 μ wide.....
A. Emini P. Henn.

b. Abhymenial hairs less than 1 mm. long; medullary layer more than 100 μ wide.....c

c. Abhymenial hairs 450 μ or longer; medullary layer not less than 250 μ wide, composed of a dense zone of hyphae oriented parallel with the surface, with a wider, more loosely arranged zone adjoining it on either side.. *A. polytricha* (Mont.) Sacc.

c. Abhymenial hairs less than 250 μ long; medullary layer less than 250 μ wide, zonation not as above.....d

d. Abhymenial hairs 70–80 μ long; medulla central to slightly abhymenial, composed of a single dense band of hyphae having a wider, more loosely arranged zone adjoining it on either side..... *A. fuscosuccinea* (Mont.) Farl.

d. Abhymenial hairs 210 μ or shorter; medulla always central, hyphae not arranged in a single dense band.....e

e. Abhymenial hairs 85–110 μ long; medulla composed of two narrow, dense bands of hyphae oriented parallel with the surface, separated by a narrower zone of loosely arranged hyphal elements....*A. tenuis* (Lév.) Farl.

e. Abhymenial hairs about 200 μ long; medulla a wide, loosely arranged, highly gelatinous band of hyphae oriented parallel with the surface, merging abruptly with the subcompact layers.....*A. cornea* Fr. ex Endl.

f. Hymenial layer at first smooth, becoming externally reticulate-porose to merulioid; context composed of a loose reticulum of hyphae whose individual elements are clearly distinguishable and not arranged in discrete parallel bands.....*A. delicata* (Fr.) Henn.

f. Hymenial layer externally smooth to venose; context always more compact than above, with hyphae frequently parallel.....g

g. Abhymenial hairs 500 μ or longer; medulla inconspicuous or lacking..... *A. mesenterica* Pers.

g. Abhymenial hairs 450 μ or shorter; medulla weakly differentiated.....h

h. Abhymenial hairs 425–450 μ long; hymenial layer 60–75 μ wide..... *A. ornata* Pers.

h. Abhymenial hairs 160 μ or shorter; hymenial layer 150–165 μ wide...i

i. Fructification resupinate with fimbriate but free margins; abhymenial hairs 65–80 μ long, 2–3 μ wide.....*A. peltata* Lloyd

i. Fructification not resupinate; abhymenial hairs 85–110 μ long, 5–6 μ wide.. *A. auricularis* (S. F. Gray) Martin

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NOTES ON BOLETES. VIII [1]

WALTER H. SNELL, ESTHER A. DICK AND L. R. HESLER

The following collections by the last author in eastern Tennessee and the Great Smokies appear to be new or interesting.

TYLOPILUS FERRUGINEUS (Frost) Sing. subsp. **vinaceogriseus** subsp. nov.

A typo carpophoro parviore, pilei colore vinaceogriseo, atque stipite graciliore differt.

Differs from the typical form in the following characters: smaller size, pileus Brownish Drab to Vinaceous Drab (Ridgway); stipe 5–10 cm. long, 8–14 mm. thick, 22 mm. at the base, reticulate only at the very apex.

In oak woods, Cades Cove, Tenn. Herb. Univ. Tenn. *17087;* Bolete Herb. WHS *1967* (type).

Xerocomus rubrosquamulosus sp. nov.

Pileo convexo, sicco, cum squamulis erectis, fibrillosis, rubro-brunneis tecto supra superficiem subtomentosam, flavam, in margine minute squamuloso et flaviore, 4–5 cm. lato; carne alba flavotincta; hymenophoro adnato, circum stipitem subdepresso, galbano, cyanescente; poris concoloribus, 1 mm. minusve latis; stipite flavo, minutis, rubrobrunneis flocculis praedito, 2.5–5 cm. × 5–6 mm.; sporis ellipsoideo-subfusoideis, hyalinis vel pallidis, 9–14.5 × 3.8–5 μ.

Pileus convex, tufted-squamulose, contracted-tufted or punctate-squamulose with fibrillose interconnections on the matted-tomentose base, toward the margin less tufted-squamulose and more minutely and finely squamulose, the squamules reddish-brown on a yellow ground-color, with the center having a reddish-brown appearance and the margin more yellow; flesh thick, soft, white with a yellow tint, unchanging, odor slight, taste mild; hymenophore adnate, somewhat depressed around the stipe, greenish-yellow changing to blue where bruised; tubes 7 mm. long; pores angular, up to 1 mm. broad near the stipe and smaller outward; stipe tapering down-

[1] Contributions from Brown University and the Botanical Laboratory of the University of Tennessee, N. Ser. 122.

ward, coarsely reticulate in the upper third, reddish-brown-furfuraceous, yellow or yellowish, paler toward the base, 2.5–5 cm. long, 5–6 mm. thick; spores nearly hyaline or very pale-colored, ellipsoid-subfusiform, 9–14.5 × 3.8–5 μ, few as small as 9 μ and only a few as large as 14.5, mostly 11–12 × 4–4.5 μ; cystidia fusoid, or ventricose-apiculate or subfusoid-apiculate, hyaline, 35–40 × 7–11 μ.

Under hardwoods, mostly oaks. Ball Camp Pike near Knoxville, Tenn. Herb. Univ. Tenn. *12710;* Bolete Herb. WHS *1968* (type).

This species is distinctive in its small, reddish-brown, tufted-squamulose or more or less erect-squamulose clothing which gives an almost scabrous appearance in the central portion of the pileus, and its greenish-yellow hymenophore.

Leccinum brunneo-olivaceum sp. nov.

Pileo convexo, e viscido subviscido, e minute subtomentoso glabro, colore Light Brownish Olive, 5–9 cm. lato; carne flavidula, rubrotincta fracta; hymenophoro paene libero, flavo; poris e subrotundis subangularibus; stipite sursum attenuato, ubique furfuraceo-scabro, subconcolori vel pallidiore cum flocculis flavulis vel ferrugineis, 4–8 cm. × 7–12 mm.; sporis in cumulo olivaceo-brunneis, ellipsoideo-subfusoideis, pallidis, 10–13 × 3.5–4.5 μ.

Pileus convex, viscid or subviscid at least in part, glabrous in the center, minutely subtomentose toward the margin, perhaps subtomentose all over at first and becoming glabrous, Light Brownish Olive, 5–9 cm. broad; flesh thick, yellowish, with reddish stains when cut, odor and taste mild; hymenophore nearly free, yellow, unchanging; pores concolorous, subrotund to subangular, small (2–3 to a mm. when dried); stipe tapering upward, furfuraceous-scabrous throughout, subconcolorous or paler with the scurfiness amber to rusty, within yellowish, stained reddish, 4–8 cm. long, 7–12 mm. thick; spores olivaceous-brown in deposit, elliptic-subfusoid, pallid or perhaps very pale yellowish, 10–13 × 3.5–4.5 μ, mostly 11–12 × 4 μ; cystidia not found; scabrosities consisting of hyphal ends and clavate dermatocystidia.

Caespitose or gregarious in mixed woods, Mt. LeConte, Great Smokies National Park, Tenn. Herb. Univ. Tenn. *13868;* Bolete Herb. WHS *1969* (type).

This species appears to be a *Leccinum* of the section Luteo-scabra of Singer. The nature of the trama could not be determined with certainty, although it appeared to be bilateral-divergent,

but the size of the pores, the shape and scabrosity of the stipe and the make-up of the scabrosity appear to be in character.

Pulveroboletus melleoluteus sp. nov.

Pileo fortasse viscido, plerumque sicco, primo subtomentoso et pulverulento, deinde glabro vel subpruinoso, fortasse minute fibrilloso-punctato, interdum in maculis subtomentoso et rarissime puverulento, primo Primuline Yellow, deinde e Honey-Yellow Chamois, 2–7 cm. lato; carne pallido-lutea, paululum cyanescenti fracta; hymenophoro adnato, paululum depresso circum stipitem, primo albo, deinde e pallido-luteo luteo, postremo Deep Seafoam Green, fortasse viridescente contusa; poris parvis; stipite apice basique e compacte tomentoso flocculoso-tomentoso, aliubi glabro, plus minusque concolori; sporis in cumulo Light Brownish Olive, elliptico-subfusiformibus, ad apicem angustatis, pallido-viridiluteis, 7–11(12) × 3.5–4.5 μ.

Pileus convex to plano-convex, up to 7 cm. broad, often only 2–3 cm.; surface when very young minutely subtomentose (a thin trichoderm) and somewhat pulverulent, when older hardly subtomentose except perhaps in spots on the margin but glabrous or subglabrous to subpruinose or possibly minutely fibrillose-punctate (the trichoderm matted down) and only rarely pulverulent, when mature viscid when wet but dry in dry weather, at first Primuline Yellow, then Chamois to Honey Yellow; flesh pale yellow, turning slightly blue when cut, odor and taste mild; hymenophore convex, adnate, slightly depressed in a narrow ring around the stipe, white when very young then pale yellow, yellow when mature becoming Deep Seafoam Green when bruised but not at all blue; tubes up to 8 mm. long; pores concolorous, very small, 3–4 to a mm. when dry, possibly 2 to a mm. when mature; trama bilateral-divergent (*Boletus*-type); stipe equal or perhaps somewhat enlarged at base, often curved, even, matted-tomentose to floccose-tomentose at apex and more or less so at base, in between glabrous with perhaps a few minute fibrils, concolorous to pale yellowish, apex often white, base yellow-mycelioid, within yellow, 2.5–6 cm. long, 5–12 mm. thick; spores Light Brownish Olive in deposit, elliptic-subfusiform, narrowed at the apex, very pale greenish-yellow, 7–11 × 3.5–4.5 μ, a very few 12 μ, mostly about 9 × 3.8 μ; cystidia fusoid with a blunt tip, carotiform, mucronate-ventricose-fusoid or ventricose-rostrate with a short tip, hyaline or perhaps filled with oily substance, 50–65 × 8–11 μ.

In mixed hemlock, pine and hardwoods. Cades Cove, Tenn. Herb. Univ. Tenn. *17993;* Bolete Herb. WHS *1970* (type).

This species belongs apparently in Singer's section Flavovelati;

it apparently has a yellow veil, the flesh turns more or less blue and the tubes become pale greenish but not blue. It is much like a small *P. Ravenelii* without an annulus, but it does not remain so yellow and the pileus does not have any testaceous tints, the tubes do not turn blue, and the spores are somewhat smaller and not so broad.

BOLETUS PULVERULENTUS Opat. forma **reticulatus** form. nov.

Two collections made in 1949 were conspicuous by their turning a dark blue everywhere upon the slightest bruising or exposure of the inner portions by cutting or breaking. Of the few species known to possess this extreme, so-called sensitivity within and without, only one was indicated by the general appearance of the specimens—*B. pulverulentus*—but the stipes were definitely reticulate. Careful studies showed, however, that these specimens were apparently just that—*B. pulverulentus* with reticulate stipe. Except for the reticulation, descriptions of the fresh and dried specimens recently found agree in precise detail, macroscopic and microscopic, with published descriptions and our own (with one oversight), the dried specimens accurately resemble the collections of the senior author, and chemical reactions of the dried specimens agree. The one oversight referred to is the failure of most, if not all, published descriptions to mention the turning of the pileic surface to deep blue when bruised, although most comments on the species emphasize the complete sensitivity. We know of no mention, however, of even a slight tendency toward reticulation of the type of this species.

The variant specimens treated here were found on lawns in Knoxville. Precise data upon the mycorrhizal associate and other desirable ecological information are not available. Accordingly, for lack of basis for proper or better taxonomic treatment, these specimens are described as a form.

A typo stipite reticulato differt.

On an old sod yard near *Robinia* and *Ligustrum* and not far from *Ulmus* but with no accurate indication of mycorrhizal associate. Knoxville, Tenn. Herb. Univ. Tenn. *19314;* Bolete Herb. WHS *1983* (type).

It may be noted that the reticulate stipe of this form is out of character with any section in which the typical form has ever been placed. At least this appears to be as true of Singer's treatment [2] as of any of the others, if we understand the situation correctly. Singer places the typical form in the section Subpruinosi of *Boletus*. The characterizations of this section (p. 38) and of the Luridi (p. 56) are given as those of the key on page 21, where the choice referring to these sections states (along with other characterizing phrases) that the pores are red if the stipe is reticulate, which apparently refers to the Luridi only, since none of the species of the Subpruinosi given have reticulate stipes, although one species (*granulosiceps*) has an indistinctly rugose-subreticulate or ribbed stipe and concolorous pores, and another species (*Weberi*) has red pores and non-reticulate stipe.

BOLETUS FULVUS Peck

Another collection (Herb. Univ. Tenn. *13848*) by Hesler near Mt. LeConte in the Great Smokies was at first thought to be a new species but we are now convinced that it is *Boletus fulvus* Peck. Peck apparently described this species on notes by McIlvaine and did not see any specimens, since he did not describe the spores. On one or two occasions two of us have found specimens which seemed to be covered fairly well by Peck's description but Hesler's specimen is the first one that fitted as closely and looked so much like McIlvaine's colored illustration.[3]

Peck described the pileus as rimose-areolate and our specimens are not, but neither is McIlvaine's drawing. The surface of some of our specimens is, however, patchy as in McIlvaine's illustration and some others also show rivulose or dendritic, raised rugulosities. In addition, the flesh of our specimens is not known to assume reddish tints as Peck stated, and the stipes, given as concolorous (that is, tawny-yellow), were rather dingy-whitish and perhaps tinged somewhat reddish. These differences do not seem to be important. Even in our present specimens the surface of the pileus is variable. As to the stipe, the mealiness is variable in

[2] Singer, Rolf. The Boletoideae of Florida. The Boletineae of Florida with notes on extralimital species III. Amer. Midl. Nat. **37**: 1–125. 1946.

[3] One Thousand American Fungi, pl. CXVI, fig. 3, opp. p. 420. 1900.

abundance and intensity of color, and the dingy whiteness of our specimens could be a faded condition, if not a variation, of the tawny-yellow concolorousness of Peck's description and McIlvaine's illustration.

Otherwise, the description fits the collector's notes in detail, and the dried specimens, with the exceptions noted, could be the specimen from which McIlvaine's drawing was made—the general form, the tawny-yellow color of the pileus with the pinkish-cinnamon or darker patches, the dark-brown marginal edge, the greenish-yellow tubes, the apical striation of the stipe (a little less pronounced in our dried specimens), the mealiness of the stipe, the basal portion of the stipe, and even the color of the stipe, which without any doubt could have been more yellow in a fresher condition.

The spores of our specimens were smoky-olive or olivaceous-brownish in deposit, subcylindrical to elliptical-subfusiform, pale-colored or perhaps very pale yellowish-olivaceous, 10–15 × 3.5–4.5 μ, mostly 11–12 × 4 μ.

As one considers this species, one is bound to wonder if the occasional collections which seem to be or to closely resemble what Peck called *fulvus* are variations of *B. flavissimus* (Murr.) Murr. All of them have been certainly very close to *B. flavissimus* but have not had the flesh and tubes turning blue or the stipe with any shade of red within at the base. Singer (*loc. cit.*, p. 64) states that both of these characters are not constant.

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AN ISOLATE OF DICTYUCHUS CONNECTING THE FALSE-NET AND TRUE-NET SPECIES [1]

T. W. JOHNSON, JR.[2]

(WITH 14 FIGURES)

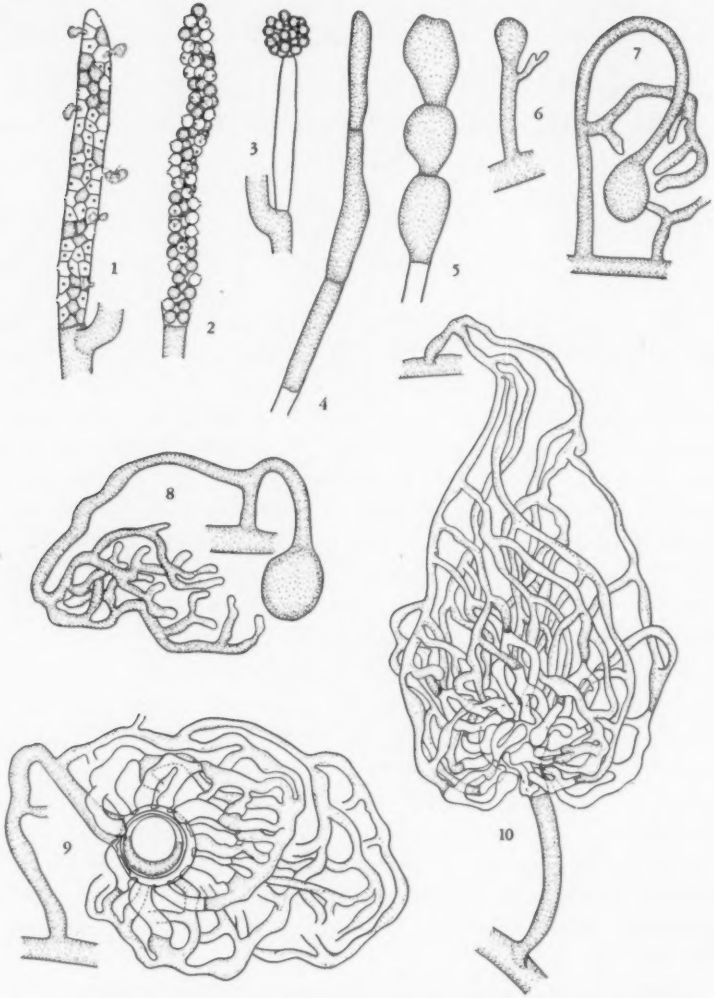
The present-day interpretation of the genus *Dictyuchus*, and of the primary separating characteristics of the species in the genus, is largely due to the investigation of Couch (**6**). Essentially, Couch's publication was instrumental in dividing the genus into three groups of species on the basis of a) the type of cyst-wall net left by the zoospores after emergence from the zoosporangium, and b) whether or not achlyoid zoosporangia were formed, or could be induced. The distinction between "true-net" and "false-net" zoosporangia in *Dictyuchus*, and a simple method for determining which type is present in a given species, is thoroughly discussed by Couch (**6**, p. 226).

In the report on his studies, Couch maintained that any strain of *Dictyuchus* which regularly had true-net zoosporangia had never been induced to produce achlyoid zoosporangia, and conversely, only those species with false-net zoosporangia regularly produced the achlyoid type, or could be induced to form such structures. A recent isolation of an unidentified, monoecious (homothallic) strain of *Dictyuchus* forms the basis of evidence which not only refutes such a separation of species, but which also presents further indication of the close kinship of *Achlya* and *Dictyuchus*.

During the course of extensive investigations in connection with a monographic study of the genus *Achlya*, a soil sample from

[1] Contribution No. 934, from the Department of Botany, University of Michigan.

[2] The author wishes to express his sincerest appreciation to Mrs. T. W. Johnson, for assistance in the preparation of the line drawings, to Mr. R. L. Wilbur, for supplying the soil sample, and to Mr. R. K. Lampton for the photomicrographs.



Dictyuchus 521. FIG. 1. An incompletely discharged, true-net zoosporangium. FIG. 2. An incompletely discharged zoosporangium of the false-net type. FIG. 3. A discharged, achlyoid zoosporangium. FIGS. 4, 5. Gemmae types. FIG. 6. An immature oogonium with a developing androgynous antheridial branch. FIG. 7. A later stage in development of an oogonium, showing immature androgynous and monoclinous antheridial branches. FIG. 8. Oogonial initial and developing androgynous antheridial branch. FIG. 9. A mature oogonium with its attendant androgynous antheridial

Florida yielded the isolate of *Dictyuchus* herein described as *Dictyuchus* 521. The fungus was recovered from the soil on hempseed "bait," and, following the usual well-known procedures for isolation of the water molds, was cultured as a single spore isolate. The description of the fungus is based on such single spore cultures propagated in 30 cc. of sterile, charcoal-filtered, distilled water, incubated at 22° C.

The conspicuous feature of the Florida isolate is the profusely-branched, contorted, umbratiform antheridial branches (FIGS. 9–14). In the majority of the oogonia, these branches appear as a "pseudoparenchymatous" mass completely covering the oogonium, and in many cases making the details of the oogonial wall indiscernible (FIGS. 10, 11). Furthermore, the abundant pitting of the oogonial wall, and the occasional irregular inner surface of the wall are in themselves distinctive characteristics. An essential feature of *Dictyuchus* 521 is the presence of true-net (FIG. 1), false-net (FIG. 2), and achlyoid zoosporangia (FIG. 3), on the same colony. The antheridial branches are predominantly of androgynous origin (arising from the oogonial stalk) (FIG. 9), or of monoclinous origin (arising from the same hypha as the oogonium to which attached) (FIG. 7, in part). Only infrequently are these structures of diclinous origin (FIG. 10). A description of the fungus follows:

Dictyuchus 521. FIGS. 1–14

Mycelium limited, diffuse, 1–1.5 cm. in diameter in the two-week old colony; extensive, dense near the substratum, 2.4–3.0 cm. in diameter in the four-week-old colony; principal hyphae moderately stout, much-branched at base, sparingly-branched at periphery of colony. Gemmae abundant, filiform, fusiform, or pyriform, rarely doliform; terminal or intercalary, single or catenulate. Zoosporangia abundant, fusiform or clavate, occasionally filiform; straight, occasionally curved or bent, infrequently angular, rarely branched; 70–462 μ long × 10–44 μ in diameter, predominantly

branches, a portion of which are not figured. Note the pitting under the large, irregular antheridial cells, and the slightly irregular inner surface of the oogonial wall. FIG. 10. An oogonium with the typical, profusely-branched, umbratiform antheridial branch of diclinous origin. All figures made with aid of camera lucida. FIG. 9, × 357; all others × 243.

127–201 × 17–21 μ; renewed sympodially. Zoospore discharge dictyoid, leaving a true-net zoosporangium, occasionally leaving a false-net one; infrequently achlyoid. Oogonia abundant, lateral, occasionally terminal; spherical, pyriform, or subglobose, occasionally oval, infrequently obovate, very rarely irregular or angular; 24–49 μ, predominantly 30–36 μ in diameter; immature oogonia rarely proliferating; oogonia commonly clustered on hyphae. Oogonial wall smooth, pitted under the antheridial cells; inner surface occasionally irregular. Oogonial stalks ½–12 times the diameter of the oogonium; curved, bent, rarely straight; usually irregular; occasionally branched, bearing 2–5 oogonia. Oospheres usually maturing. Oospores eccentric, spherical, very rarely ellipsoid; single, extremely rarely two in number; 19–36 μ, predominantly 26–28 μ in diameter. Antheridial branches androgynous or monoclinous, infrequently diclinous; stout, profusely branched, contorted or irregular, usually umbratiform; not persistent. Antheridial cells large, irregular or contorted, simple or branched; persistent; laterally or apically appressed, or attached by wide, short or long, cylindrical, ovoid, or globular projections; fertilization tubes usually formed. Mature oospores germinating by short, slender, unbranched germ tubes, bearing small, filiform, true-net, dictyoid zoosporangia.

From mud, Cypress swamp pool, Highlands Hammock State Park, Lebring, Florida, August 13, 1950; legit. R. L. Wilbur.

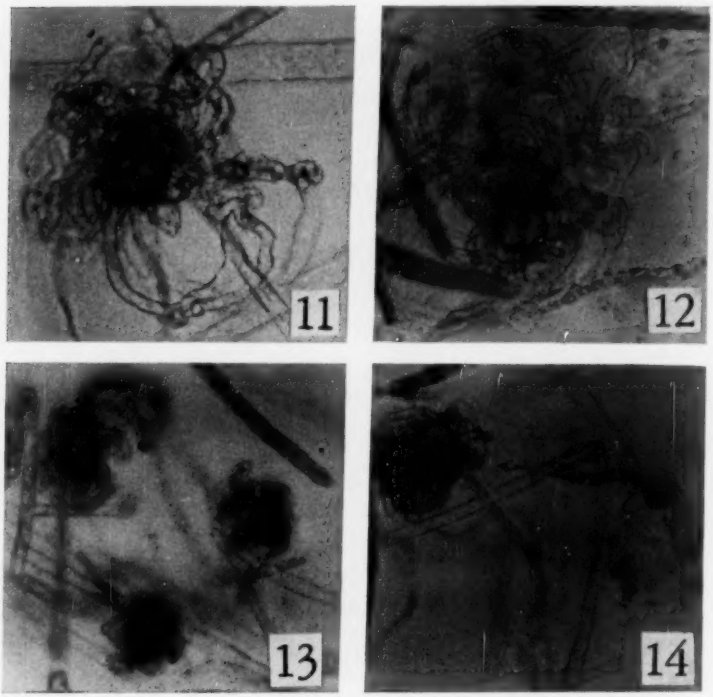
The true-net type zoosporangia are predominant in *Dictyuchus* 521, with the false-net and achlyoid types appearing in varying abundance from sub-culture to sub-culture. The false-net type becomes increasingly noticeable as the colony ages, while the achlyoid zoosporangia are most abundant in the younger cultures (4–6 days old). The possibility that *Dictyuchus* 521 might not be a single spore isolate naturally suggests itself in view of the various zoosporangial types. However, single spores from all three types of zoosporangia, when germinated on corn-meal agar, and subsequently transferred to hempseed in water cultures, matured into similar plants. The profuseness of the antheridial branches around the oogonium and its attendant stalk frequently made it impossible to determine the origin of those branches. Consequently, immature oogonia and antheridia (FIGS. 7, 8) generally had to be observed to determine this characteristic.

Dictyuchus monosporus Leitgeb (**8**), *D. anomalus* Nagai (**9**), *D. achlyoides* Coker and Alexander (**3**), and *D. pseudoachlyoides*

Beneke (**1**) have been available as living material for comparison with the present fungus. Aside from rather close similarities in size of oospores and oogonia (which is held to be of little taxonomic significance), our fungus is obviously distinct from these known species. *Dictyuchus missouriensis* Couch (**6**) is much nearer to *D. anomalus* Nagai than it is to *Dictyuchus* 521. From the description of *Dictyuchus pseudodictyon* Coker and Braxton (**4**), one would be led to assume that the much-branched, irregular, tortuous antheridia, which "almost entirely" enwrap the oogonia, are suggestive of the Florida isolate, but the figures of antheridial branches of *D. pseudodictyon* (**4**, *Pl. 15, figs. 2, 4, 5*) do not even remotely resemble those commonly found in *Dictyuchus* 521. The large, irregular antheridial cells which often cover the oogonium in *Dictyuchus carpophorus* Zopf (**10**) resemble the oogonia and attendant antheridial cells in older cultures of *Dictyuchus* 521 where the antheridial branches have disappeared, leaving an irregular mass of persistent antheridial cells still attached to the oogonial wall. The profuse, short, lateral outgrowths from the hyphae of *D. carpophorus* (**10**, *Pl. 2, fig. 1*), however, are not found in our isolate. In the final analysis, if Couch's basic separation of species on the type of discharged zoosporangium is accepted, *Dictyuchus* 521 is entirely separable from any of the previously described species in the genus by nature of its possession of true-net, false-net, and achlyoid zoosporangia.

The characteristics of *Dictyuchus* 521, then, seem sufficiently distinct to warrant the erection of a new species, were one inclined to do so. There are, however, certain considerations which must first be taken into account. In the first place, the later-formed oogonia of *Dictyuchus* 521 are not always associated with the profusely-branched antheridia, but rather, possess short, branched antheridia which are more typical of those illustrated for *D. pseudodictyon* (**4**, *Pl. 15, fig. 2*). This suggests a relationship of our fungus to *D. pseudodictyon*, the closeness of which may be borne out by a study of further isolates representing strains of *Dictyuchus* 521 and Coker and Braxton's fungus. It would be fallacious to overlook the possibility of as yet unisolated strains which would link up these two species, with the present one, perhaps, exhibiting the extreme

of strong antheridial branch development. The author's present studies in the genus *Achlya*, especially in the *prolifera* group of the sub-genus *Euachlya*, have repeatedly borne out this close overlapping of species, and the existence of numerous intermediate forms between what have previously been assumed to



FIGS. 11–14. Photomicrographs of *Dictyuchus* 521, showing the characteristic contorted, profuse antheridial branches. All figures approximately × 100.

be valid species. It is not inconceivable, and is indeed more than likely, that such a condition exists in other genera of the Saprolegniaceae.

Secondly, the genus *Dictyuchus* has not been thoroughly studied, and in fact, very few isolates of known species have been reported by investigators other than the authors of those species. The synonymy in *Dictyuchus monosporus* is a case in point.

Coker and Matthews (**5**), following Coker's original suggestion (**2**, p. 157), reduced *Dictyuchus carpophorus* Zopf (**10**) to synonymy under Leitgeb's species. It is true that Zopf's description, while extensive, was lacking in details which would be important in subsequent identification of the species. Nevertheless, it is questionable that *D. carpophorus* should have been so reduced to synonymy under Leitgeb's heterothallic species, when Zopf clearly illustrated his plant with monoclinous antheridial branches (**10**, *Pl. 2, fig. 1*). Such an antheridial branch origin presumably does not occur in a strictly bisexual species. *Dictyuchus anomalus* Nagai, likewise, was reduced to synonymy under *D. monosporus*. Nagai described his fungus as completely lacking in antheridia, and this feature has been borne out consistently in several collections of this species made by the author (**7**). *Dictyuchus missouriensis* Couch, while also lacking in antheridial branches, does differ from *D. anomalus* in its production of false-net zoosporangia. These two species are not at all different in the formation of achlyoid zoosporangia, since we have observed several isolates of *D. anomalus* with the true-net type of zoosporangia which it normally has, but in which the achlyoid type is also formed. These isolates of *D. anomalus* likewise contradict Couch's statement concerning the occurrence of zoosporangial types (**6**, pp. 225–226). Aside from the lack of false-net zoosporangia in *D. anomalus*, and minor differences in oogonium and oospore size in Nagai's fungus and Couch's isolate, the two are remarkably similar. Granted that, in the past, false-net, true-net, and achlyoid zoosporangia showed a marked correlation in consistent appearance on a given species, *Dictyuchus* 521 now exhibits evidence that the occurrence of these sporangial types is not as strictly defined as Couch reported. With these considerations in mind, the fact suggests itself that there is no more a valid reason for considering *D. anomalus* a synonym of the heterothallic species, *D. monosporus*, than there is for considering *D. missouriensis* as a species distinct from *D. anomalus*. It is believed that further evidence gleaned from a study of a considerable number of isolates representing *D. anomalus* and *D. missouriensis* would do much toward clarifying the taxonomy in these closely-allied species.

Finally, it is obvious from the description of *Dictyuchus* 521 that there no longer exists in this genus the clear-cut separation of species on the basis of the type of zoosporangium exhibited by a given species. For these reasons, we have chosen not to describe *Dictyuchus* 521 as a new specific entity at this time.

The present paper does not profess to solve the taxonomic problem in *Dictyuchus*. There is, however, a strong indication, based solely on the characteristics exhibited by *Dictyuchus* 521 and *D. anomalus*, that the specific entities included in the genus are not as clearly defined as might be suspected. Until an intensive investigation of the species in this genus can be carried out, any "new" species might well be considered doubtful.

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A NEW LEAF SPOT OF CELASTRUS SCANDENS L., THE CLIMBING BITTERSWEET

CARL W. BOOTHROYD

(WITH 1 FIGURE)

A bittersweet vine grown as an ornamental near a home in Ithaca, N. Y., was found diseased by a leaf-spotting fungus in late summer of 1948. The fungus proved to be a typical *Marssonina* of the anthracnose group and subsequent investigation revealed that it was common on *Celastrus scandens* L. in the vicinity of Ithaca in 1948 and 1949 (FIG. 1).

No record of a *Marssonina* on *C. scandens* could be found in the literature. Examination of exsiccati material of the same plant in some twenty phanerogamic and cryptogamic herbaria throughout the United States and Canada was also negative.

A *Marssonina* has been reported several times in this country on another member of the Celastraceae, *Evonymus atropurpurea* Jacq. That *Marssonina* has been identified as *M. thomasiana* (Sacc.) Magn.; the first report, as far as can be determined, being that by H. S. Jackson of an Indiana collection in June, 1916.

The Marssonina leaf spot on *C. scandens* closely resembled that on *E. atropurpurea*. Size, shape, and color of diseased spots on both suscepts were almost identical. The two-celled hyaline spores from *C. scandens* leaves were somewhat larger than those from *E. atropurpurea*, but that difference was small and no greater than the variation noted between spore measurements from several collections of each individual suscept.

However, it was unusual that such a striking leaf-spotting fungus should be fairly common on *E. atropurpurea* in the midwest and not upon the widely distributed bittersweet, *C. scandens*. It was also odd that the disease should be so common upon bittersweet in the vicinity of Ithaca, and yet fail to show up upon *E.*

atropurpurea of which there are several plantings nearby, although that plant species is considered rare in the northeast.

FIG. 1. *Marssonina* leaf spot of bittersweet, *Celastrus scandens* L. Note lesions on fruit.

Attempts to inoculate seedlings of each plant species under natural conditions have to date substantiated the above observation. *C. scandens* became heavily spotted, while *E. atropurpurea* remained free of infection.

It is possible that two distinct forms of *M. thomasiana* are present; one pathogenic upon *C. scandens,* the other upon *E. atropurpurea.* Further studies are being made of this new leaf spot on bittersweet.

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NOTES AND BRIEF ARTICLES

The Poroid Genus Diacanthodes Singer

This monotypic genus was published in Lloydia **8**: 141. 1945, with *Daedalea philippinensis* Pat. as its type and rough spores as its chief distinguishing feature. Except for the rough epispore Dr. Singer would very probably have been contented with *Abortiporus* Murr. One might wonder if *Grifola Berkeleyi* and *Elfvingia lobata* are to be segregated from their obvious congeners because of their rough spores; or whether genera like *Lactarius, Russula, Inocybe* and *Cortinarius* are to be dissected *ad libidum* because of the presence or absence of spiny spores or cystidia of various shapes and sizes.

As to *Daedalea philippinensis,* Mr. J. A. Stevenson wrote me recently, "Even Lloyd was unfamiliar with it although he was often in Paris. In an unpublished manuscript on the Polyporaceae of the Philippines he discusses the species in his appendix of unknown species as follows:

'*Polyporus philippinensis.* Named as *Daedalea philippinensis* but closely related to *Polyporus rufescens* (Stip. Pol. p. 157) which has daedaloid pores but is generally classed as *Polyporus.* It has echinulate spores and is close and I judge from description the same as *Polyporus asterosporus* of Brazil (Note 282)'."—W. A. Murrill.

The relative dates of Gray's Natural Arrangement, Mérat's Nouvelle Flore and Hooker's Flora Scotica

Shortly after the completion of an earlier note on "The Relative Dates of S. F. Gray's Natural Arrangement and Fries's Systema" [1] an entry was encountered in the English Catalogue [2] giving for the

[1] Mycologia **33**: 568–570. 1941.

[2] Peddie, R. A., and Waddington, Q. English catalogue of books . . . 1801–1836. p. 241. 1914.

Natural Arrangement the date "Jan. [18] 22." A sentence to that effect was submitted for insertion in the note, but arrived too late for inclusion. Since it was the date of Gray's work relative to that of Fries's that was under discussion, rather than the precise date, and the relation was not altered by the possibility of a later date for Gray, no notice of the additional information was published.

Quite recently it became clear that the nomenclature of fungi must take into account the second edition of Mérat's *Nouvelle Flore*,[3] a work embodying not only the mycology but also the nomenclature of the French masters Bulliard and DeCandolle. Thence soon arose the necessity of a choice between a name of Gray's and one of Mérat's. Fortunately a precise date for the latter is on record;[4] Mérat's work was registered during the week June 9–15, and quite certainly antedates Gray.

It is not yet possible to regard any date for the *Natural Arrangement* as established. In a footnote to an article signed on December 31, 1821, Greville[5] cites Gray's work. Although alterations in Greville's paper may well have been possible in 1822,[6] this citation justifies the suspicion that the *Natural Arrangement* may have been published on its ostensible date, November 1, 1821, or at any rate later in that year, rather than in January, 1822. Other published information is equally inconclusive. The Edinburgh Review lists Gray's work in its "Quarterly list of new publications, from October 1821 to January 1822"; since the next such list is "from February to June 1822"[7] this listing does not exclude the possibility of publication in January; here "to" means "through." In the Literary Gazette for November 3, 1821, appears over the name of Gray's publisher an announcement beginning with the words: "New botanical work. In a few days will be published, A Natural Arrangement of British Plants. . . ." In the number for January 19, 1822, Gray's book is listed under

[3] Mérat, F. V. Nouvelle flore des environs de Paris. 2 ed. Vol. 1, Cryptogamie. 1821.

[4] Bibliographie de la France **10** (24): 320. 1821.

[5] Wernerian Nat. Hist. Soc. **4**: 71. 1822.

[6] On a preceding page ([viii]) is a notice dated May 1822.

[7] Edinburgh Review **36**: 569. 1822; **37**: 275. 1822.

the heading "Books published this day."[8] From some of the accompanying notices, and from the fact that the list appeared only weekly, it is clear that "this day" cannot be understood literally; the listing does not even mean "books published *by* this day," since one so entered is announced only for future publication; the entry is an advertisement, not an editorial notice. In the New Monthly Magazine for November 1, 1821, Gray's work is listed under the heading "Nearly ready for publication"; in the number for January 1, 1822, it is entered under "New publications."[9] If the latter has any value as evidence, it indicates publication before January 1st, in contradiction of the "Published this day" entry for the 19th.[10] One of these listings (or perhaps more than one) probably constitutes the basis for the *English Catalogue* date, rather than any special information available to its compilers. Because of the conflict and uncertainty of the evidence, it seems necessary[11] to treat Gray's names as published on the date borne by his book. Perhaps there is evidence in Great Britain—such as letters by S. F. Gray or J. E. Gray or their enemies, persecutors, and slanderers—from which to decide the question.

In the English Catalogue (p. 280) the Flora Scotica[12] is entered as having been published in "May [18]21." No other date appearing, that given by Peddie & Waddington ought to be accepted. Hooker's mycology is, "for the most part, taken from the *Synopsis Methodica Fungorum* of *Persoon*." The order of publication in 1821 of the works in question is, then, *Systema mycologicum* (January 1), *Flora scotica* (May), *Nouvelle Flore* 2 ed. (June 9–15), *Natural Arrangement* (November 1).—DONALD P. ROGERS.

[8] Literary Gazette, and Journal of Belles Lettres, Arts, Sciences, &c. **1821** (250): 704. 1821; **1822** (261): 48. 1822.

[9] New Monthly Magazine and Literary Journal (London) [ii] **3** (11): 583. 1821; **6** (13): 26. 1822.

[10] For the information supplied by these three literary journals I am indebted to the kindness and proficiency of Mr. James Tobin, reference librarian of the New York Public Library.

[11] Art. 36 bis, adopted at Stockholm: ". . . In the absence of proof establishing some other date, the date given in the work must be accepted. . . ."

[12] Hooker, W. J. Flora scotica. 1821. (Acotyledons, part 2, pp. [3]–162.)

REVIEWS

MEDICAL MYCOLOGY, edited by Frederick Reiss. Annals New York Academy of Sciences **50**: 1209–1404. 1950. Price $2.75.

The New York Academy of Sciences has published 15 papers which were presented at a conference on medical mycology sponsored by the Section of Biology of the Academy. Dr. Frederick Reiss, Consulting Editor and Conference Chairman, in a short introduction, outlines the limitations of our knowledge in this field and the importance of new problems which are emerging. This series of papers occupies 196 pages and covers such a variety of subject matter that limitations of space will permit little more than a catalog of contents.

Dodge, Carroll W. (Mycological research and the progress of medicine) reviews the early history of medical mycology and suggests the need for further studies of possible relationships between susceptibility to fungus invasion and endocrine dysfunction.

Salvin, S. B. (Public health aspects of fungus infections) points out that although systemic mycoses are relatively rare they are responsible for 0.3% of deaths in the United States in 1945, and that this is more than the total of deaths due to rabies, smallpox, relapsing fever, leprosy, brucellosis, paratyphoid fever, plague, cholera and anthrax. The diagnosis of a mycosis is made more difficult by the fact that certain fungi which are capable of causing pulmonary disease may, under other conditions, be present in sputum as contaminants without etiologic significance. The natural habitats and modes of transmission of actinomycosis, sporotrichosis, coccidioidomycosis and histoplasmosis are discussed. The most frequent fungus infection, dermatophytosis, poses important problems of control and therapy, especially in the case of epidemic ringworm of the scalp in children.

Moore, Morris (Evaluation of classification of pathogenic fungi) discusses the problems of classifying pathogenic fungi and the effect of medical and other environmental factors on variability. The closely related fungi of chromoblastomycosis were cited to illustrate

the divergent views of those who would place them in two or three species of a single genus as opposed to those who would distribute them among 5 genera.

Conant, Norman F. (Future developments in mycological investigative methods) discusses the trend toward fundamental studies of nutrition, physiology, chemistry and immunology of the fungi. Metabolic products of fungi are being used widely in studies of the epidemiology of mycoses.

Weidman, Fred D. (Superficial dermatomycoses caused by Trichophytons, Microsporums, and Epidermophytons) states that the dermatophytes occur only on man and animals and that although moisture, friction, endocrine balance, and local pH influence susceptibility to infection there are immunologic factors which are not understood. The histopathology of the lesions in the epidermis and the general principles of laboratory diagnosis and treatment are described.

Carrión, Arturo L. (Chromoblastomycosis) presents a monographic treatment, reflecting some 20 years of his meticulous investigations, of chromoblastomycosis. He accepts as authentic 159 cases, of which 80% were in the tropics or subtropics. Several clinical types and their histopathology are described. Case histories indicate that the soil is the natural habitat of the fungi causing this disease. The infection is essentially superficial and usually involves the foot. Therapy, except for radical surgery, is usually ineffective. The author lists the synonymy of the fungi, assigning them tentatively to a genus not generally accepted as valid, and presents a schematic arrangement of varieties of the principal species.

Christie, Amos (Histoplasmosis and pulmonary calcification) outlines the historical development and experimental bases for his belief that there is a prevalent non-fatal form of histoplasmosis which results in pulmonary calcification. Three case histories are reviewed. Survey studies have shown varying degrees of correlation between histoplasmin skin sensitivity and the presence of pulmonary calcification. Histoplasmin is non-specific and interpretation of the skin reaction to it depends upon comparative tests with other fungus antigens. The evidence from the coincidence of geographic distribution of the two phenomena is reviewed.

Benham, Rhoda W. (Cryptococcosis and blastomycosis) reviews in detail the differential characteristics, nomenclature, biology and serology of two pathogenic fungi and the symptomatology and epidemiology of the two diseases they cause and the historical basis for their confusion which her earlier work had resolved.

Georg, Lucille K. (The nutritional requirements of the faviform Trichophytons) investigated the vitamin deficiencies of *T. album, T. discoides* and *T. ochraceum* and concludes that these are varieties of one species which she calls *T. faviforme.* Growth on whole grain natural media and on a basic medium to which inositol and molecular thiamine were added is characterized by formation of conidia and macroconidia. A few strains of these or related species are autotrophic but did not produce spores on the basic medium. *T. equinum* requires nicotinic acid or nicotinamide. The minimal amounts of essential vitamins were determined for several strains. *T. schoenleini* is differentiated from *T. faviforme* by its clinical manifestations and its autotrophic metabolism.

Gougerot, Henri (New insight gained in general pathology and practical medicine by the study of sporotrichoses) reviews the history of sporotrichosis, the synonymy of the fungus, *Sporotrichum schenckii,* and the very extensive studies by himself and associates of this mycosis in France. He reports isolation of the fungus from plants in 1908.

Robbins, William J. (Growth requirements of dermatophytes) reviews work work published from his laboratories with a strain of *Trichophyton discoides* which requires molecular thiamine, pyridoxine and inositol, and a strain of *T. mentagrophytes* with no vitamin deficiencies, but which is stimulated by addition of several amino acids. Some of the sterile mutants which regularly appear in *Trichophyton* seem to have developed new enzyme systems. Hydroxyproline inhibits growth of dermatophytes.

Peck, Samuel M. (Fungus antigens and their importance as sensitizers in the general population) reviews a part of the very extensive literature on the allergy of the dermatophytoses. He defines the criteria for diagnosing a trichophytid and discusses the types and incidence of this allergic lesion. The dermatophytes present an important public health problem, particularly in epidemics of ringworm of the scalp in children and in industry where "athletes'

foot" may be aggravated by working conditions. Demonstration that some of the dermatophytes produce small amounts of a penicillin-like antibiotic offers a rational explanation for the occurrence of penicillin sensitivity in persons who have not previously received penicillin therapy.

Martin, Donald S. (Practical applications of immunologic principles in the diagnosis and treatment of fungus infections) states that the fungi causing systemic mycoses are weakly antigenic and discusses the serology of blastomycosis. Although the blastomycin skin test is not a generally useful diagnostic procedure a positive test is an indication for desensitization before treatment is begun. Agglutination and complement fixation tests are also erratic in blastomycosis.

Iams, Alexander M. (Histoplasmin skin test) reviews the problem of histoplasmin sensitivity in relation to pulmonary calcification, the non-specific nature of histoplasmin and the geographic distribution of histoplasmin sensitivity and reports finding only 1% of a series of children in Minneapolis sensitive to this antigen.

Archibald, Reginald M. and Frederick Reiss (Some biochemical implications from a study of growth of pathogenic fungi on media containing single amino acids) reported that most amino acids tested, when present singly, are able to support growth of 13 fungi pathogenic for man. Amount of subsurface growth is inversely proportional to the ability of an amino acid to support growth.

The influence of errors in spelling in one or two reference books is reflected in the misspelling in two of the papers of *Microsporum* and in one of *Hormodendrum* and *Sporotrichum*.

This series of papers presents a very useful summary of the present knowledge of several important aspects of Medical Mycology.—C. W. EMMONS.

PLANT BIOCHEMISTRY, by James Bonner. Pp. 537. New York, The Academic Press, Inc. 1950. $6.80.

Plant biochemistry as a field of investigation is again becoming popular now that the emphasis is upon the dynamics instead of upon the statics of the subject. The enzymatic processes of formation of a few essential substances are considered more important than the listing of all of the organic compounds found in plants.

This book is written chiefly about the higher green plants from the current view-point. The gaps in our knowledge of the chemistry of plants are numerous and some are large. When such gaps in information are encountered, Bonner fills them as best he can by reasoning by analogy from the biochemistry of bacteria, animals, *Neurospora,* and that favorite microbe of the animal biochemist, yeast. For example, the pathways of amino-acid syntheses and interconversions were worked out with *Neurospora* mutants and the resulting concepts applied to the higher plants, for which the details are meager. The incompleteness of our knowledge of plant chemistry is not surprising. The workers are few; the subject is large. Bonner is one who has contributed much to several fields of plant biochemistry during the last twenty years.

The book is divided into six parts: Carbohydrates and Carbohydrate Metabolism, The Cell Wall and Cell Wall Metabolism, Plant Acids and Plant Respiration, Metabolism of Nitrogenous Compounds, Secondary Plant Products, and Certain Aspects of Plant Growth. Photosynthesis and Plant Growth Substances (auxins not vitamins) are the subjects of the last section. Inorganic biochemistry is largely neglected.

Although the expert will find each chapter inadequate because obviously in a book of this size and scope much must be omitted, the student for whom the book is intended will find it an excellent introduction to the subject. The numerous structural formulae and diagrams of the course of reactions contribute to the clarity of presentation. The subject index is good. The book is well printed.

Minor errors were detected such as the use of "bacteria" for the singular form. The red absorption band of chlorophyll-a (p. 469) has a most unusual overhang. The references are cited in numerical order so that much time must be wasted if one wants to know what other papers by a particular author are cited. A more serious criticism is the author's use of "the case of" many too many times. This particular phrase, though it may be permissible to the physician, the lawyer, and the social worker, decreases clarity of expression when used, as it is here, in discussing chemical processes. This and other examples of sloppy writing which could

have been removed by proper editing should not have been allowed to mar an otherwise excellent book.

All who are interested in the physiology and biochemistry of plants, white and green, will want to have this book within easy reach of his working desk.—FREDERICK KAVANAGH.

A MONOGRAPH OF CLAVARIA AND ALLIED GENERA, by E. J. H. Corner. xv + 740 pp., 298 figs., 16 plates. Annals of Botany Memoirs No. 1. Oxford University Press, London. 1950. Price $21.00.

The necessity for a radical revision of the classification of the Basidiomycetes has long been recognized and substantial progress has been made in that direction during recent years. An acceptable system is not yet in sight, but the publication of Corner's imposing monograph of the clavarioid fungi represents notable progress. Heterobasidiomycetes are not considered, but all known genera of homobasidiomycetous fungi with clavate or coralloid fructifications, except *Thelephora,* are discussed in detail; all known species are described, many very completely, from first-hand study, others less adequately because of the faulty original descriptions and the unavailability of type or authentic material. A large number are illustrated, either by superb line drawings or colored figures or both. The few familiar genera represented by *Clavaria, Lachnocladium, Typhula* and *Pistillaria* are here expanded to twenty-seven, some of the additional genera being older and little-known creations of other students; nine are proposed as new. A number of subgenera and many new species, varieties and forms are proposed. These are all listed for convenient reference under "Novitates" on pp. 690–701. There are numerous new combinations which may be found by referring to any known name in the comprehensive alphabetical list of species at the end. The genera and, within each genus, the species are arranged in alphabetical order, making it a simple matter to turn to the description desired.

No family name is used. It is the author's contention that the family Clavariaceae as ordinarily recognized exhibits too great confusion to have any taxonomic significance. The classification is based on microscopic study of the mycelium and the hymenial elements of the fructification and on the development of the fruiting

body. To those accustomed to the more familiar and certainly more superficial methods which have served until now, the techniques will seem unduly difficult and confusing and the distinctions obscure. It may confidently be predicted, however, that the adoption of these methods will lead to a much clearer understanding of the larger fungi.

The cost of the book is high, but not unreasonably so considering the extensive text, the difficult character of the printing and the number and quality of the illustrations. The book is a necessity for any laboratory where serious study of the higher fungi is being attempted.—G. W. M.

COMMON BRITISH FUNGI. Elsie M. Wakefield and R. W. G. Dennis. v–ix + 289 pp. 111 color plates. P. R. Gawthorn Ltd., London, England. 1950. 35 s. net.

This is a relatively non-technical book designed for general use. It covers the larger Basidiomycetes, including the Heterobasidiae (exclusive of rusts and smuts) Aphyllophorales, Agaricales and Gasteromycetes. The descriptions cover macroscopic features well, and give some information on microscopic characters. It is a book which will be used by specialist and amateur alike. The treatment of the gill fungi is conservative. The classification of the gill fungi is along Friesian lines, but with the Cantharellaceae as a separate family. From the discussion presented by the authors it is clear that they regard this as the best possible compromise to serve the group which will use the work most, and that it does not necessarily represent their views as professional mycologists. They are to be congratulated on the format, readability, and serviceable binding. The single outstanding feature, however, is the inclusion of 111 color plates in a work of so modest a price. Such a work as this at double the price could not be published here under present conditions.

On the deficit side the following criticisms appear to need mentioning. The introductory material has been reduced to the point where it is inadequate. Certain emphatic statements are made which were not actually necessary and which some may regard as controversial, *i.e.*, on page 14 it is stated that the mycelium of fungi possesses no cellulose in its membranes. On page 13 the

wording is such, in the comments on the manufacture of carbohydrates, that water is not included as a constituent of a carbohydrate. Also, p. 19, the statement is made that in *Hygrophorus* the development is gymnocarpic. This is not true for all species, as all who have worked with the subgenus *Limacium* probably know from their own observations.

The color plates, though generally good, are not all up to the same standard. In this respect the authors have had the same bad luck as others of us who have tried to illustrate fungi in color. The illustrations of *Cortinarius violaceus, C. semisanguineus* and *C. cinnamomeus* are examples of this. The illustration of *Psathyra subatrata* (Pl. LXXX) applies to a different species, possibly one undescribed. I naturally object to the inclusion of the species of *Cystoderma* in the genus *Lepiota*. *Cystoderma*, by virtue of containing species with adnate gills, is a logical genus in the Friesian system, or would be merged with *Armillaria*. The name *Psalliota* was doubtless used because of the established custom in Britain.

The inclusion of drawings of the spores of species of *Lactarius* and *Russula* is a feature which will be of great help to all who study these genera. However, I wish, personally, that in other genera more emphasis had been placed on characters of the cystidia and the elements making up the cuticle of the pileus. This would have made the work even more usable to those outside the British Isles.

In spite of these shortcomings it is a book all students of the Basidiomycetes should have.—ALEXANDER H. SMITH.

DISEASES OF CEREALS AND GRASSES IN NORTH AMERICA (FUNGI EXCEPT SMUTS AND RUSTS), by Roderick Sprague. 538 pp. The Ronald Press Co., New York. 1950. Price $7.00.

With the aroused interest among workers in plant pathology in the development of better forage crops, this summary of the diseases of cereals and grasses is a very welcome addition to the literature in that field. Three hundred and eighty-four "species and subcategories of species" occurring on the mainland of North America and Central America as well as Hawaii are treated. The West Indian species are not included. Each entry is described with its host range and to these is appended a brief discussion of

its pathological implications. As the result of the author's wide field experience, the descriptions and discussions of the treated species are very complete and, because of his first-hand knowledge, the most valuable part of the book.

The organization of the material is a compromise between a strictly pathological and a strictly mycological treatment and like most compromises falls short of accomplishing its purpose. The diseases are arranged under the fungus names and the main chapters are: Phycomycetes, Ascomycetes, Basidiomycetes and Fungi imperfecti. Within these groups the species are gathered alphabetically; first by generic names, then by species. Identification of unknown material would be difficult, for the keys are not clear. For example, *Polymyxa* is in the section of the Phycomycetes with "Mycelium lacking" while *Olpidium* is in the section "Mycelium scanty but usually evident." Again, in the Ascomycetes, *Claviceps* is placed in the section "Perithecia in black stroma."

The volume concludes with a glossary of the more technical terms and an excellent bibliography of more than two thousand titles.—JOSEPH P. GILMAN.

MANUSCRIPT

Publication in MYCOLOGIA is restricted to members of the Mycological Society of America. The Board reserves the right to alter this regulation should circumstances warrant. When a paper has two or more authors, the person submitting the paper is expected to comply with this requirement.

Papers should be submitted in duplicate to the Editor-in-Chief or to any member of the Editorial Board. Papers will be published in the approximate order of their approval except for the address of the retiring President, which will be published when received, and papers whose cost of publication is paid by the authors, which will be run at excess pagination. Notes, Brief Articles and Reviews may be used at the discretion of the Editor-in-Chief.

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